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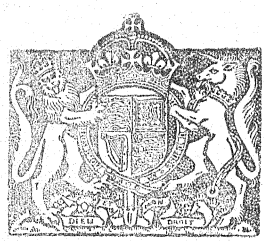

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ORIGINAL ARTICLES

THE VACCINATION OF HIGHLY SUSCEPTIBLE ANIMALS AGAINST RINDERPEST

By J. R. Haddow and J. A. Idnani, Imperial Veterinary Research Institute, Mukteswar

(Received for publication on 17 June 1946)
(With two text-figures)

Edwards [1927] by attenuating and fixing rinderpest virus in goats has provided India with a reliable and safe means of vaccinating plains cattle. The larger problem being solved, the demand for providing for the more susceptible populations of the hills, for buffaloes, particularly those in milk or in-calf, and for sheep and goats has become more insistent. This demand has been accentuated by the wider movement of these animals necessitated by the war. Vaccination with goat virus alone in these species gives rise to an unpleasantly large number of severe reactions, which may even be followed by deaths varying in extent with local conditions. The use of small doses of serum to damp down such reactions is now almost universal, but this is expensive and unwieldy when large herds or flocks have to be protected. The annual reports of this Institute contain many references to attempts at simplifying the procedure, but none of these has proved acceptable to field workers. It was decided at the beginning of the war that the position should be reviewed and fresh efforts made to find a satisfactory solution. Entirely satisfactory results have not been attained, but progress in certain directions was achieved and this is now recorded.

Three different strains of virus were available for experiment, (1) line E, a highly virulent epidemic ox strain causing about 90 per cent mortality in hill-bulls, (2) line D, and old laboratory passaged strain of ox origin causing about 12 per cent mortality, (3) line W, the goat-fixed strain causing about 12 per cent mortality. As buffaloes were seldom available, experiments were made on hill-bulls, the mortality in which is considerably higher than in buffaloes.

Alumina-gel vaccine

Haddow and Idnani [1941] reported that fowls could be vaccinated against Ranikhet disease with a virus adsorbed on alumina-gels. Small-scale experiments were carried out on similar lines on goats using vaccines made separately from line E and line W viruses. Six goats were vaccinated with each type of vaccine. Only three in each group became available for test and all were found to be solidly immune. The work however, was not continued, because the difficulties of preparing and transporting such a vaccine under field conditions in India would clearly be almost insurmountable.

Formalized vaccine

Formalized vaccines prepared with line E and line W viruses were also tried on a small scale in goats. These were prepared somewhat on the lines of Daubney [1928]. Spleen pulp was formalized 1 : 1000 in nutrient broth and stored at 5°C. Vaccine prepared with line E virus was tested after three and seven days on six goats each time, and that prepared with line W was inoculated into six goats after six day's storage. No alteration of the viruses was perceived and owing to the high cost of such a vaccine the work was dropped.

Glycerolized vaccine

Since the year 1918 when Kakizaki initiated the method, several workers have made trails with glycerolized vaccine. Bennett [1936] reported a series of experiments carried out in the Sudan.

Infected spleens collected at the height of reaction were minced and to this twice the quantity (by weight) of 60 per cent glycerol was added. This product was then aged either at 37°C. or at 0°C. Bennett concluded that glycerolized spleen pulp was rendered safe for vaccination purposes by storage either for three days at 37°C. or for three months at 0°C. Stress was laid on dosage—the fresher the vaccine the smaller the dose but 1 gram of spleen pulp per 100 kilograms live weight was considered suitable. This author also stated that vaccine after treatment at 37° C. could be stored for many months in the cold and used in the field without loss of antigenic power. Pfaff [1938] has examined the subject of vaccination with glycerolized virus.

Vaccination of hill-bulls. Our experiments were designed in a similar manner to those of Bennett. Line E virus was employed to infect bulls and spleens were collected in every case on the fifth day. Twice the weight of 60 per cent sterilized glycerol was added to the minced tissue. The mixture was stored at 37°C. and inoculations were made from it at various intervals. Table I shows the results of experiments on bulls, weighing about 100 kilograms.

TABLE I

Glycerolized vaccine (line E) in hill-bulls

Period of storage	No. of Animals	Dose of vaccine suspension	Reaction	Tested after days	Result of test
37° C. for 24 hours	1	3·0 c.c.	*nil*	17	I
	1	do.	do.	17	Slt R; S
37°C. for 48 hours	1	do.	do.	16	R; D
	1	do.	do.	16	Slt R; S
37°C. for 72 hours	1	do.	do.	15	R; S
	1	do.	do.	15	I
37°C. for 72 hours followed by storage in refrigerator for 7 days	1	do.	do.	27	R; D
	1	do.	Reacted and died of rinderpest; day 11*		
for 14 days . .	2	do.	*nil*	20	I
for 21 days . .	2	do.	do.	13	R; D

Slt R=Slight temperature reaction; R=reaction; I=Immune; D=Died; S=Survived;
*Possibly an accidental infection

It would appear from Table I that the vaccine was rendered safe for use after only 24 hour's storage at 37°C., but with the dosage employed it failed to impart complete or consistent immunity; also that the vaccine lost its potency completely after three weeks in cold storage.

To elucidate further the viability of the virus used for preparing the vaccine, spleen tissue was emulsified in double its weight of distilled water, stored at 37°C. in flat containers and tested at the same dose rate at 24, 48 and 72 hours. One of the two bulls tested with material stored for 24 hours developed rinderpest and died on the tenth day. Its companion showed no reaction but developed rinderpest on immunity test. All the remaining animals reacted on test with virulent virus. This experiment indicated that rinderpest virus in the form of thick suspensions is very variable in potency when stored at 37°C.

In view of the fact that, line E virus was of highly infectious nature and therefore not likely to be safe for widespread field use, further work was prosecuted with the less virulent virus, line D. Vaccine was prepared in exactly the same way from line D, except that the period of ageing at 37°C. was kept at two days. The results are shown in Table II.

TABLE II

Glycerolized vaccine (line D) in hill-bulls

Period of storage	No. of animals	Dose of vaccine suspension	Reaction	Tested after days	Result of test
37°C. for 48 hours	1	3·0 c.c.	*nil*	12	Slt R; S
	1	do.	do.	12	R; D
	2	30·0 c.c.	do.	12	I
37°C. for 48 hours, followed by storage in refrigerator for 7 days	1	3·0 c.c.	do.	10	I
	1	do.	do.	10	R; D
	1	30·0 c.c.	do.	10	I
	1	Died of other causes			
for 14 days	2	3·0 c.c.	do.	12	I
	2	30·0 c.c.	do.	12	I
for 21 days	1	3·0 c.c.	do.	14	R; S
	1	do.	do.	14	I
	2	30·0 c.c.	do.	14	I
for 28 days	1	3·0 c.c.	do.	14	R; S
	1	do.	do.	14	R; D
	2	30·0 c.c.	do.	14	I

The results indicate that the line D vaccine was safe for use after two days' ageing at 37°C. and that it retained its antigenic value for four weeks, when administered at the higher dose rate, which alone gave solid immunity.

The period of ageing of the vaccine was then further reduced to 24 hours in one lot and 30 hours in another. The result of this experiment is given in Table III.

TABLE III

Glycerolized vaccine (line D) in hill-bulls

Period of storage	No. of animals	Dose of vaccine suspension	Reaction	Tested after days	Result of test
37°C. for 24 hours	1	3·0 c.c.	*nil*	23	R; D
	1	do.	do.	23	I
	2	30·0 c.c.	R; D		
37°C. for 30 hours	2	3·0 c.c.	*nil*	23	Slt R; S
	2	30·0 c.c.	do.	23	I
37°C. for 24 hours, transported 90 miles in 2 days on ice	11	3·0 c.c.	Slt R.	2 months	3 tested; I
				12 months	4 tested; I
37°C. for 30 hours transported 90 miles in 2 days on ice	11	30·0 c.c.	Slt R.	2 months	1 tested; I
				12 months	8 tested; I

In this experiment, as well as in previous tests, it became apparent that two important factors needing control are the period of ageing and the size of the dose. After the vaccine has been rendered safe, a fairly large dose has to be employed to ensure protection.

Another brew of glycerolized vaccine was now prepared with line D virus and tested after 18, 24 and 48 hours at 37°C. (Table IV).

TABLE IV

Glycerolized vaccine (line D) on hill-bulls

Period of storage	No. of animals	Dose of vaccine suspension	Reaction	Tested after days	Result of test
37°C. for 18 hours	2	3·0 c.c.	Slt R.	25	I
37°C. for 24 hours	2	30·0 c.c.	do.	25	I
	1	3·0 c.c.	R; D.		
	1	do.	Slt R.	25	I
37°C. for 48 hours	2	30·0 c.c.	do.	25	I
	1	3·0 c.c.	*nil*	24	R; D
	1	do.	do.	24	Slt R; S
	2	30·0 c.c.	do.	24	I

The results shown in Table IV confirm the finding that at least 30·0 c.c. of glycerolized vaccine heated for 48 hours at 37° C. have to be employed to ensure protection.

Vaccination of goats. Vaccines were prepared with line E and line W viruses. A few tests were also made on hill-bulls.

TABLE V

Glycerolized vaccines in goats (line E) and hill—bulls (lines E and W)

Period of storage	No of animals	Dose of vaccine suspension	Reaction	Test after days	Result of test
Vaccine prepared with line E virus—test in goats					
37° C. for 48 hours	1	1·5 c.c.	*nil*	20	Slt. R; S
	1	do.	do.	20	D
	2	7·0 c.c.	Slt R	20	I
	1	15·0 c.c.	*nil*	20	I
37°C. for 48 hours followed by storage in the refrigerator for 7 days	1	do.	Slt R	20	D, other causes
	1	1·5 c.c.	*nil*	22	Slt R; S
	1	do.	Slt R	22	D
	1	7·0 c.c.	do.	22	I
	1	do.	D, other causes		
	1	15·0 c.c.	*nil*	D; P	
	1	do.	do.	22	I
for 14 days	1	1·5 c.c.	Slt R; D; P		
	1	do.	*nil*	15	Slt R; S
	1	7·0 c.c.	D, other causes		
	1	do.	*nil*	15	I
	1	15·0 c.c.	*nil*	15	I
	1	do.	Late R	15	I
for 21 days	1	1·5 c.c.	*nil*	17	D
	1	do.	do.	17	D; P. P
	2	7·0 c.c.	do.	17	I
	1	15·0 c.c.	do.	17	R; S
	1	do.	do.	17	D, other causes
for 28 days	1	1·5 c.c.	do.	23	D; P
	1	do.	R. D. other causes		
	2	7·0 c.c.	*nil*	23	I
for 28 days	1	15·0 c.c.	D, P. P		
	1	do.	*nil*	23	Slt R; S
Vaccine prepared with line E virus—test in bulls					
37°C. for 72 hours	1	3·0 c.c.	Died of other causes		
	1	do.	*nil*	20	D. other causes
	1	do.	Slt R	20	do.
	3	30·0 c.c.	*nil*	20	I
Vaccine prepared with line W virus—test in bulls					
37°C. for hours 72	1	3·0 c.c.	*nil*	20	Slt R; S
	2	do.	do.	20	I
	3	30·0 c.c.	do.	20	I

Slt R=Slight temperature reaction ; P=Pneumonia ; PP =Purulent pertonities

These results, considered as a whole, confirm the findings of Bennett, except that higher doses are required for completely satisfactory results in susceptible hill-cattle and goats. No opportunity for field trials presented itself, but we are of the opinion that for general purposes the method would prove too expensive in goats, and by analogy possibly also in buffaloes although this species was not available for experiment.

A further observation during the course of routine work was that line E or line D viruses, when inoculated directly into goats or sheep, gave rise to much milder reactions that when goat virus was used. In fact, in these animals it appeared that line D virus could be used as a vaccine. Thus, out of six goats and four sheep inoculated directly with line D virus, all showed a mild thermal reaction and, except one goat which died of other causes, all survived. Field trials are to be arranged.

RABBIT VIRUS VACCINE

Edwards [1924] reported that rinderpest could be transmitted to rabbits, but unfortunately the strain obtained by him died out before final conclusions could be drawn as to its vaccinating properties.

In earlier years, [Haddow (unpublished)] numerous unsuccessful attempts were made to confirm Edwards' work, using line D and the fixed goat strain. Morcos [1931] tried to infect guinea-pigs, rabbits, white rats and dogs with rinderpest virus, by feeding or inoculation. No attempt was made to extend the scope of the experiments by transferring the virus from these animals into bovines. No laboratory animal used by him is reported to have contracted rinderpest. Inoue [1934] passaged a laboratory strain of bovine rinderpest virus to rabbits up to the 50th generation. Nakamura, Wagatuma and Fukusho [1938] carried out extensive systematic studies on the effect of serial passage of rinderpest virus in rabbits. They concluded that the virus was reduced in virulence for cattle, and after the 100th passage test calves often survived after developing more or less distinct symptoms.

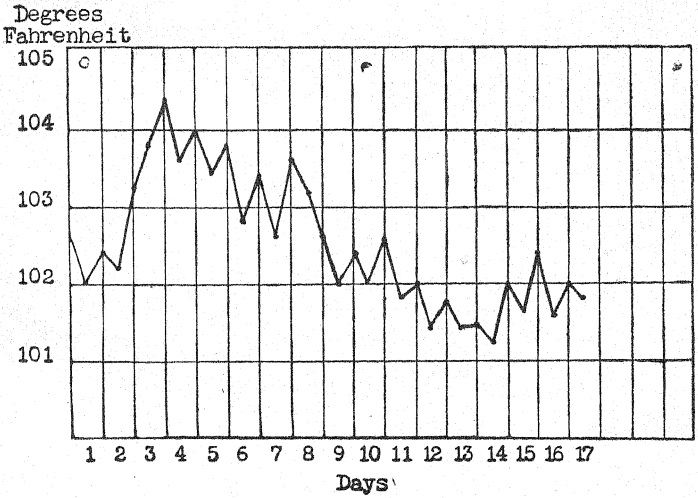

FIG. 1.—*Rabbit passage 59.*

When a newly-isolated epidemic strain became available to us, fresh efforts were made to passage it in rabbits. In two attempts success was immediately achieved. Transmission was started by inoculating 5 c.c. blood subcutaneously and intra-venously to two pairs of rabbits. The pair inoculated intravenously gave the most marked reaction. Three cubic centimetres of pooled blood from all four rabbits was subinoculated subcutaneously into a pair of rabbits. Subsequent passages were carried out with pooled blood subcutaneously into two rabbits. As with previous successful attempts, the only symptom in the rabbit was a rise of temperature on third or fourth day, which was not very marked in the first few passages but later was always distinct (Fig. 1). The temperature fell after two to four days and the rabbits invariably recovered. As the work progressed, it was discovered that rabbits four or five months old are much the best material for passage, older rabbits giving variable reactions. The virus may persist in the blood for fourteen days and in the spleen for twelve days, though the virus is not present in large quantity. Simultaneously we repeated the experiments with lines D and W, but with entirely negative results. We are therefore of the opinion that, if transmission to rabbits is to be successful, it is essential to use a fully virulent strain.

The results of tests on bulls, goats and buffaloes are recorded in Table VI.

TABLE VI

Vaccination of bulls, goats and buffaloes with rabbit virus

No. of passage and material	Time of collection material and period of storage	Dose	Animal and Number	Reaction to first inoculation	Immunity test result
1 Mixed blood	Fresh	1 c.c.	1 bull	R; D	
2 Mixed blood	22 days	1 c.c.	1 bull	*nil*	Naturally immune
3 Mixed blood	18 days				
	14 days				
3, 3 blood	Fresh, bled days 3 & 4 resp.	1 c.c.	2 bulls	R; D	
4, 5 blood mixed	Fresh, bled day 32 & 28 resp.	2 c.c.	2 bulls	*nil*	R; D
8 Spleen	fresh 5th day	1 c.c. 1 : 100	bull	R; S.	I
		do.	bull	R; D	
30 spleen	fresh 5th day	do.	bull	R; D	
40 spleen	fresh 5th day	do.	bull	Slt R.	I
50 do.	do.	20 c.c. 1 : 100	bull	R; D	
50 do.	5th day spleen stored 9 days in refrigerator	do.	bull	*nil*	R; D
55 do.	fresh 5th day	5 c.c. 1 : 100	bull	Slt R.	I
	do.	do.	bull	do. (Destroyed)	
60 spleen	fresh 5th day	5 c.c. 1 : 100	2 bulls	Slt R.	I
70 do.	do.	5 c.c. 1 : 100	bull	do. spleen taken 9th day	

TABLE VI—*contd*

Vaccination of bulls, goats and buffaloes with rabbit virus

No. of passage and material	Time of collection of material and period of storage	Dose	Animal and Number	Reaction to first inoculation	Immunity test result
70 spleen	fresh 5th day	5 c.c.	goat	taken of spleen 9th day	I
70 do.	do.	1 : 100	goat	do.	Died before test
$\frac{?}{1}$ Bull spleen	9th day spleen stored 4 days in refrigerator	1 c.c. 1 : 10	calf	do.	I
$\frac{?}{1}$ Bull spleen	Stores 4 days in refrigerator	1 c.c. 1 : 10	calf	do.	I tested after 6 months
$\frac{?}{1}$ do.	stored 13 days	10 c.c. 1 : 10	bull	do.	I
$\frac{?}{1}$ do.	do.	1 c.c. 1 : 10	5 goats	*nil*	R
80 Spleen	fresh 5th day	1 c.c. 1 : 10	3 calves	Slt R.	I. Tested after 6 months
80 do.	do.	1 c.c. 1 : 100	3 calves	do.	i. I. 2. I. Tested after 6 months
80 do.	do.	1 c.c. 1 : 1000	3 calves	do.	I
84 do.	do.	1 c.c. 1 : 10	2 goats	do.	i.I. i.P.
84 do.	do.	1 c.c. 1 : 100	2 goats	do.	I
84 do.	do.	1 c.c. 1 : 1000	2 goats	*nil*	R
86 do.	do.	1 c.c. 1 : 500	5 calves	Slt R.	I
90, 92 do.	do.	1 c.c. 1 : 100	3 bulls	do.	I
90, 92 do.	do.	1 c.c. 1 : 10	2 kids	do.	I
90, 92 do.	do.	1 c.c. 1 : 100	2 kids	do.	I
90, 92 do.	do.	1 c.c. 1 : 1000	2 kids	do.	I
93 do.	do.	1 c.c. 1 : 500	calf	do.	I
102 do.	do.	1 c.c. 1 : 500	2 calves	do.	I
105 do.	do.	do.	3 calves	do.	I
115 do.	do.	do.	2 calves	do.	I
115 do.	fresh 7th day	do.	2 calves	do.	I

TABLE VI—*contd*

Vaccination of bulls goats and buffaloes with rabbit virus

No. of passage and material	Time of collection of material and period of storage	Dose	Animal and Number	Reaction to first inoculation	Immunity test result
115 spleen	fresh 12th day	1 c.c. 1 : 500	2 calves	*nil*	R
115 do.	fresh 15th day	do.	2 calves	do.	do
122 do.	fresh 12th day	1 c.c. 1 : 10	2 calves	Slt R.	I
126* do.	fresh 5th day	1 c.c. 1 : 100	11 buffaloes (in milk) & 10 goats	do.	I
139 do.	fresh 7th day	1 c.c. 1 : 500	2 calves	do.	awaiting tests
154 do.	fresh 5th day	1 c.c. 1 : 20	2 calves	do.	I
154 do.	do.	1 c.c. 1 : 200	1 calf	do.	Piroplasmosis probably I.
154 do.	do.	1 c.c. 1 : 1000	1 calf	do.	I
154 do.	do.	1 c.c. 1 : 2000	1 calf	*nil*	R
161 Sent in live rabbit for field trial					
174 Liver & spleen	fresh 5th day	1 c.c. 1 : 20	1 calf	*nil*	I
174 do.	do.	1 c.c. 1 : 200	1 calf	do.	R
174 do.	do.	1 c.c. 1 : 2000	1 calf	do.	R
174 do.	stored 5 days in refrigerator	1 c.c. 1 : 20	1 calf	do.	do.
174 do.	do.	1 c.c. 1 : 200	1 calf	do.	do.
174 do.	do.	1 c.c. 1 : 2000	1 calf	do.	do.
174 do.	Stored 10 days in refrigerator	1 c.c. 1 : 20	1 calf	do.	do.
174 do.	do.	1 c.c. 1 : 200	1 calf	do.	do.
174 do.	do.	1 c.c. 1 : 2000	1 calf	do.	do.
176 Sent in live rabbit for field trial.					

* Trial at Military Farm.

It will be seen from Table VI that the virus transmitted through rabbits remained virulent for bulls up to about the 50th passage, in which from the 55th passage onwards there was a definite and gradual decrease in virulence. None of the bulls inoculated after the 55th passage showed any symptom other than rise of temperature, usually rather later than normal, (Fig. 2) and this type of reaction was obtained as far as the 154th passage, after which reactions were uncertain though they remained typical in rabbits. It will also be observed that the virus does not stand storage well, even at refrigerator temperatures; reliable results could not be obtained from the fifth day onwards and in this respect rabbit virus is much more fragile than goat fixed virus. The average virus content of rabbits would also appear to be much lower; hence less dilute suspensions must be used. The results in goats were similar to those obtained in bulls.

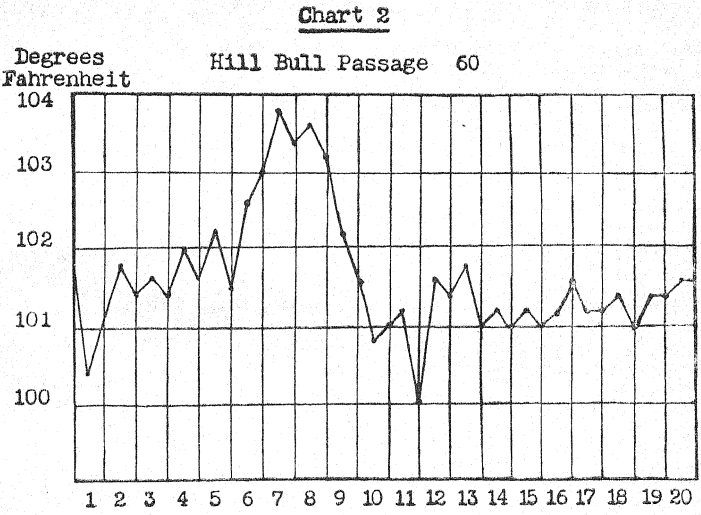

FIG. 2. *Rabbit passage 60.*

In buffaloes, three field trials were arranged in co-operation with the Military Farms.

(i) At the first of these, one of us (J. A. I.) inoculated eleven milch buffaloes and ten goats with rabbit virus from passage 126, the amount used being 1·0 c.c. of 1 : 100 suspension of rabbit spleen. The virus was taken to the place of inoculation in live rabbits. These buffaloes and goats showed slight reaction to the vaccination and all were immune on test 21 days later. There was no interference with the buffaloe's milk yield.

(ii) The second test was carried out by the Indian Military Veterinary Laboratory. From passage 161 a few serial passages were continued at that laboratory and altogether 469 buffaloes were inoculated. The dose given was 1 c.c. of a 1 : 100 spleen suspension which was used on the day of preparation. The temperature and milk yield of sixty animals were recorded for fourteen days after vaccination. No systemic disturbance was observed and milk yields were unaffected. Sixteen of these buffaloes chosen at random were tested 1—2½ months after vaccination, eight had no reaction,

six showed a more or less severe reaction and recovered, while two died of acute rinderpest. As some of these buffaloes were probably immune before vaccination, it appears that at best successful 'takes' occurred in only a small percentage.

(iii) Owing to the hot weather, the temperature reactions in rabbits at the Military Veterinary Laboratory were difficult to define and it was therefore decided to repeat the trial with fresh material from Mukteswar and to use stronger suspensions of both liver and spleen. Accordingly, the 176th passage in rabbits was sent in live rabbits, and was passaged once only at the Laboratory. Material from this passage was inoculated into:

Group 1.—40 buffaloes at rate of 1 c.c. 1 : 100 liver suspension
Group 2.—40 buffaloes at rate of 1 c.c. 1 : 10 liver suspension
Group 3.—40 buffaloes at rate of 1 c.c. 1 : 100 spleen suspension
Group 4.—40 buffaloes at rate of 1 c.c. 1 : 10 spleen suspension

The reaction in five animals of each group were recorded in detail, but unfortunately owing to an outbreak of what is believed to be buffalo-pox, the records of all but group 2 were vitiated. In group 3, three animals showed an indefinite temperature reaction and diminution in milk yield. Thirtyfive days later, three animals were chosen at random from each group and tested with virulent virus. The only reactions were that one animal in group 1 and one in group 4 had severe reactions and recovered, one animal in group 2 and one in group 4 had very slight temperature reactions. Unfortunately, experiments at Mukteswar, concurrent with the third trial, indicated that the virus had by this time lost most of its invasive power for highly susceptible cattle.

Further work on rabbit passaging is now in hand, with the object of seeing (*a*) whether passages between the 50th and 100th will regularly give a satisfactory vaccine for buffaloes, (*b*) whether the attenuated rabbit virus can be transferred to hill bulls and maintained in them.

Summary

1. Results are recorded of a small-scale experiment with virus adsorbed on alumina-gels.

2. Results of experiments on glycerolized vaccine are recorded. The findings of previous workers on the value of such vaccines are confirmed, but to obtain entirely reliable results it is necessary to increase the dosage and reduced the period of storage under glycerol. The method is somewhat expensive.

3. Successful attempts were made to fix rinderpest virus in rabbits. For this purpose, it is necessary to use a strain of high virulence for cattle. The virus becomes progressively attenuated in rabbits, until it loses all or nearly all its invasive power for buffaloes and cattle. At intermediate stages the virus appears to constitute a suitable vaccine, but the exact stage and method of maintenance are not yet defined. The virus in rabbit tissue is not present in large quantity and cannot be stored for long outside the animal body. It can, however, be readily transported in the living rabbit to areas within a range of seven days.

Acknowledgements

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SOME OBSERVATIONS ON THE IMMUNIZATION OF SHEEP AND GOATS AGAINST RINDERPEST

By H. K. Lall, B. Sc., M.R.C.V.S., Government Livestock Farm, Hissar

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OUTBREAKS of rinderpest have been observed in sheep and goats from time to time in various parts of India by Bawa [1940], Chowdhry [1941] and various other workers (personal communication). A rise in the incidence of the disease in these animals occurred during recent years. It was considered that that was due to an increase in the animal traffic to various battle fronts in and outside India. Ten outbreaks were recorded in the year 1943-44 in Ambala Division of the Punjab alone, causing very heavy mortality. Cattle were not affected in these outbreaks. It became apparent that some definite steps were required to devise methods of immunization. Anti-rinderpest serum has been tried by various field workers, with varying results in affected animals but it did not prove very effective in controlling the outbreak at Hissar. Bawa [*ibid*] reported that in goats the use of anti-serum alone was not very satisfactory, specially because of the short duration of immunity. In sheep, however, Bawa [1939] reported that the use of goatis was a success as a prophylactic against rinderpest. Srikantiah [1934] and Viswanathan [1937] have mentioned the use of anti-rinderpest serum in sheep as an essential adjunct to goatis to avoid severe reaction, but Viswanathan reports 3·6 per cent mortality from vaccination, in spite of the serum. These authors do not mention the breed of sheep used in their experiments. Gopal Singh (personal communication) tried goatis on sheep and goats as a prophylanis in face of outbreaks of rinderpest in Gurgoan district. With a view to produce immunity against rinderpest, some experiments were conducted at Hissar and the following observations were made.

In this note the term 'goatis' has been used for goat tissue vaccine; a mild reaction was determined by the fact that the animal had only a thermal reaction but normal in its behaviour and feeding and a severe one, by high temperature, loss of appetite, dullness, sluggish movement, congested mucous membranes conjunctivitis, etc. A thermal reaction means a rise in the temperature as compared to the normal temperature under similar conditions.

(A) Goats

It is well known that the goats used for the production of goatis show severe reaction on inoculation of the goat virus. It is also believed that if they are allowed to live, they succumb to the disease, or, in cases of recovery, they remain emaciated [Saunders, 1936]. A series of experiments were performed to produce immunity in sheep and goats by use of goatis alone or its following modifications.

(1) serum and goatis mixed for half an hour,
(2) goatis followed by a dose of serum 24 hours later,
(3) goatis immediately followed by serum.

The goatis in all the experiments was obtained from the Punjab Veterinary College, per messenger and efforts were always made to use it immediately after its receipt. In any case it was kept in ice till required for use. The number of the virus (*i.e.* the number of the goat at the Punjab Veterinary College, from which the goat virus was procured) and the date of arrival and despatch were noted in all the experiments. The serum used was the 'ordinary' serum obtained from the Imperial Veterinary Research Institute.

Experiment No. 1

Batch No. of the virus	157
Date of preparation of the virus	28 October, 1944
Date of experiment	1 November, 1944

Five goats of the Betal breed and five hill goats were employed in this experiment and the various observations made are tabulated in Table I.

TABLE I

Reaction of goatis alone, mixed with serum and followed by serum on Betal and Hill goats

Nos. of goats	Vaccine used	Type of reaction	Day of reaction	Duration of reaction
B 950 . . .	Goatis alone 0·01 gm. .	Mild	5th day . .	4 days
H 155 . . .	do. 0·01 gm. . .	do.	5th day . .	4 days
B 49 . . .	0·01 gm. goatis and 5 c.c. of serum mixed for half an hour and then injected	No reaction . . .	....	
B 998 . . .	do.	do.	....	..
H 76, 137 . .	do. . . .	Delayed and doubtful .	7th day . .	The day of reaction
B 104,84 . .	Goatis followed by 20 c.c. of serum the next day	Mild and delayed reaction (doubtful). This doubt was cleared by the next experiment	do. . .	do.
H 62,75 . . .	do.	No reaction	....	..

N. B.—B and H denote Betal and Hill goats respectively.

It was observed that the goatis by itself produced rather a mild reaction. The results at first sight seemed to be encouraging but rather suspicious because of the usual severe reaction obtained by injection of goatis. The suspicion was eventually confirmed as it was found that the refrigerator storing the goatis had broken down for a day and that probably the virus thus suffered attenuation. This observation suggested the use of attenuated goatis as a vaccine for the goats, but it could not be given a trial. Serum and goatis mixed together and kept for half an hour at room temperature did not produce any definite reaction. The reaction produced by inoculation of goatis followed by serum 24 hours later was mild in Betals and *nil* in hill goats. All the animals retained their normal appetite during the experiment.

Experiment No. 2

Batch No. of the virus 177
Date of preparation of virus . . . . . 20 October, 1944
Date of experiment . . . . . 24 November, 1944

As the goatis had inadvertently had been attenuated in the first experiment, another batch of 10 goats, including the five from the first experiment, was given goatis to observe under local conditions the effect of normal goatis. The effect of the injection of blood virus which was administered to three animals, was also noted. The latter was given only for the purpose of comparison. The findings are tabulated in Table II.

TABLE II

Reactions of previously vaccinated goats to goatis and of goats injected with blood virus

Nos. of goats	Vaccine used	Type of reaction	Day of reaction	Duration of reaction
950 (of previous experiment)	Goatis alone . . .	No reaction . . .	....	....
84 do. . .	do.	do.	....	....
104 do. . .	do.	do.	....	....
998 do. . .	do.	Severe reaction . .	4th day . .	3 days. Death on 4th day
603 . . .	do.	do.	5th day . .	8 days, died
862 . . .	do.	do.	do. . .	5 days
37 . . .	do.	Mild	4th day . .	3 days
49 (of previous experiment)	Blood virus . . .	Severe reaction . .	do. . .	do.
949 . . .	do.	Moderate . . .	do. . .	4 days
9 . . .	do.	No thermal reaction but very dull	....	....

Goatis produced a very severe reaction in all the goats except Nos. 950, 84 and 104 which had reacted mildly in the previous experiment. Two goats (Nos. 49, 998) which were inoculated with mixed serum and goatis in the previous experiment exhibited a severe reaction thus indicating that serum and goatis mixed had no immunizing value. Goat Nos. 603 and 998 died showing lesions of rinderpest. It appears from these observations that goatis need not necessarily cause mortality in all the injected goats and that it produces a different type of reaction in individual animals. Blood virus did not produce a reaction in any way different from that produced by goatis.

Four animals reacted on the fourth day, three on the fifth day and one (No. 9) without having any thermal reaction remained very dull for a considerable period, thus proving that the temperature was not the only criterion of the severity of the reaction. From this experiment it became clear that :

(i) Goatis by itself was of no use because of the severe reaction produced by it, and

(ii) serum and goatis mixed were also useless as their inoculation failed to produce any reaction.

Experiment No. 3

Batch No. of the virus 190
Date of preparation of virus . . . . . 8 December, 1944
Date of experiment . . . . . 9 December 1944

Thus, in order to compare the reactions of goatis and serum injected at the same time and goatis followed by serum 24 hours later another five animals (78, 91, 70, 73 and 100) were injected with 0·01 gm. goatis in 1 c.c. of saline followed by 5 c.c. of anti-rinderpest serum, half an hour later, four (16, 882, 808, 95) were inoculated with goatis followed by serum the next day and one goat (No. 859) was kept as a control (goatis alone). The results are tabulated in Table III.

TABLE III

Reaction of Betal goats to goatis followed by serum immediately and 24 hours later

Nos. of goats	Vaccine used	Type of reaction	Day of reaction	Duration of reaction
859	Goatis	No thermal reaction otherwise very dull	....	....
17	Goatis followed by 20 c.c. of serum next day	Very severe	5th day	5 days, aborted on 6th day
882	do.	Fairly severe	4th day	4 days
808	do.	do.	do.	5 days
95	do.	Mild	3rd day	do.
100 and 78	Goatis followed by 5 c.c. of serum 30 minutes later	No reaction (No. 100 got 8 c.c.)	....	....
70	do.	Severe	5th day	5 days
91	do.	Mild	4th day	3 days
73	do.	do.	do.	2 days

The control as well as the four goats which got the serum 24 hours after the injection of virus reacted severely. Four out of five injected with goatis and virus at the same time had a mild reaction and no loss of appetite was noticed in them. No. 17 aborted, while goat No. 859 was fairly dull without having any thermal reaction. The observations further reveal that serum and goatis given at the same time produce a mild reaction in Betal goats and in virtue of this some immunity is likely to be produced.

Experiment No. 4

Batch No. of the virus 253
Date of preparation of virus . . . . . 13 January, 1945
Date of experiment . . . . . 8 January, 1945

Eight *desi* (country) goats were inoculated to see if the results would be the same as that obtained in Betal and hill goats. Goat Nos. 1, 2 and 3 were injected with goatis followed by 5 c.c. of serum; goat Nos. 4, 5 and 6 got goatis followed by 7 c.c. of serum, while goat Nos. 7 and 8 were kept as controls. The following observations were made (Table IV).

TABLE IV

Reaction of vaccination in nondescript village goats

Nos. of goats	Age and sex	Day reaction	Duration of reaction
No. 1	6 teeth F	3rd day	10 days
No. 2	Old F	No reaction	..
No. 3	,, F	5th day	4 days
No. 4	4 teeth F	3rd day	5 days
No. 5	4 teeth F	2nd day	3 days
No. 6	4 teeth F	2nd day	10 days
No. 7	2 teeth F	3rd day	7 days
No. 8	4 teeth F	do	15 days

Goat No. 4 died on the 6th day with clots of blood in its abdominal cavity resulting from a rupture of the liver. The oral cavity and the digestive tract were normal; a few patches of consolidation were seen in the right lung. The rupture of the liver was presumably the cause of death and as the goat was seen fighting with others on various occasions, it is considered that the rupture was due to mechanical injury. Goat No. 6 also died and on post mortem examination gastro-enteritis and pneumonia of the left lung were observed. In this as well as in previous experiments it was noticed that the animals usually feel dull from the sixth to the eighth day after being injected (or even later), i.e., about 2 or 3 days after the reaction. In this group of animals, no variation in the reaction was observed although the dose of the serum was varied, but probably not sufficiently:

Four animals reacted on the third day
Two animals reacted on the second day and
One reacted on the fifth day.

From the duration of the reaction, it would seem that these animals were more susceptible than the Betals, but it was observed that they were feeding normally and were quite active in spite of the fever. If the rise in temperature was not taken into account, no abnormality would be noticed. The controls, however, where no serum was given, were dull and would stand against the wall with arched back in a very typical posture. It thus follows that the serum-goatis method of immunization can be used successfully in *desi nondescript* goats. The fact that the aged animals reacted in a similar manner to the young ones indicates that no appreciable amount of immunity is conferred with age.

Experiment No. 5

The batch No. of the virus . . . . . No. 322
Date of preparation . . . . . 9 March, 1945
Date of experiment . . . . . 13 March, 1945

To find out the variation in the susceptibility of hill goats and Betals, five Betals (Nos. 31, 111, 102, 879, 43) and nine hill goats (Nos. 88, 80, 79, 87, 82, 83, 81, 86, 84) were immunized by the goatis and serum method. The results are tabulated in Table V.

TABLE V

Reaction in Hill and Betal goats, showing the variation in susceptibility

Nos. of goats	Mode of virus administration	Day of reaction	Duration of reaction
B 102	Goatis followed by 5 c.c. of serum	3rd day	5 days
B 11	do.	do.	3 days
B 31	do.	do.	2 days
B 879	do.	do.	8 days
B 43	Goatis followed by 8 c.c. of serum	4th day	1 day
H 88	Goatis followed by 5 c.c. of serum 15—30 minutes later	2nd day	5 days
H 87	do.	3rd day	do.
H 83	do.	do.	do.
H 84	do.	do.	do.
H 86	Goatis followed by 8 c.c. of serum	2nd day	4 days
H 80	Goatis alone	3rd day	7 days
H 79	do.	do.	9 days
H 82	do.	do.	7 days
H 81	do.	do.	9 days

It was observed that the reaction in hill goats lasted longer than in the Betals, but these goats being more active by nature, did not exhibit any thermal sign of dullness, even when they had pyrexia. In the group of hill goats in which no serum was administered, no death occurred, but degree of pyrexia, duration of reaction and dullness were more marked. In case of Betal No. 43, which got 8 c.c. of serum, it was interesting to observe that it reacted for a day only. This observation points towards assessing of proper dosage of serum to attenuate the goatis so as to produce a mild reaction. These findings lead us to the conclusion that hill goats can also be immunized by serum-goatis method and are not as highly susceptible as the local hill goats. These animals had been born and bred at Hissar and whether that made any difference in their behaviour to goatis is difficult to decide, but the indications are that their susceptibility is reduced in plains as proved by the results of an experiment carried out at Kangra, which showed that these were more susceptible, though no strict comparison can be made because there the goats of other breeds like Betal, etc., were not employed in the experiment at the same time. Haddow in this connection [1939] has stated 'that hill goats like hill cattle are experimentally highly susceptible.' Some healthy animals including some bovines were left in direct contact with the immunized animals and were feeding and drinking from the same trough, to see their potentiality for spreading the disease and it was observed that they did not spread the disease to in-contact animals of their own species or the bovines. Thus, in villages where segregation is not always possible, there would be no danger of spread of the disease from vaccinated to the unvaccinated ones. A similar observation has been recorded by Cornell *et al* [1941] in case of bovines, though DeCosta [1937] has reported otherwise.

Immunity test

Strain of bull virus H.S. 217 . 218 E line
Date of despatch . . . 19 March, 1945
Date of experiment . . . 23 March, 1945

The bull virus of a virulent strain was obtained per messenger from Mukteswar to test the immunity of goats vaccinated with serum goatis. Four Betal goats (Nos. 100, 70, 91, 78) and four hill goats (Nos. 76, 137, 155, 75), along with three buffalo calves to act as controls, were injected with bull virus. The goats were given 1½ to 2 c.c. of bull virus per head while the buffalo calves were given 5 c.c. All the Betal goats which had given a mild or even a blocked reaction with serum and goatis inoculation failed to react to bull virus, thus proving that the serum and goatis inoculation had conferred solid immunity. The hill goats (Nos. 76, 137, 75), which had been inoculated with

serum and goatis and had not reacted at the time of vaccination, reacted to bull virus, indicating that serum and goatis mixed did not produce any immunity. Hill goat No. 155, which had mildly reacted on vaccination did not evince any thermal reaction. It will thus be observed that the Betals and the hill goats behave in a similar fashion to inoculation of serum and goatis and develop a similar degree of immunity. Here it may be mentioned that the bull virus did not produce a very severe reaction (only a thermal reaction), either in bovines or goats or sheep at Hissar. The observations in this connection made elsewhere (see section under sheep) also show that the bull virus produces a reaction milder to that of goatis in sheep and goat. Nicolle and Adilbey [1902] and Walker [1922] also noticed only thermal reaction in sheep on inoculation of bovine blood virus. The reaction in the cattle of Hissar breed is also milder [Report of the Disease Investigation Officer, Punjab, 1944-45] as compared to the reaction described from other parts of India which is probably due to the climatic conditions or the natural resistance of Hissar breed.

The results are tabulated in Table VI.

TABLE VI

The results of community test in goats vaccinated with goatis alone and in conjunction with serum

Nos. of Goat	Breed	Date of immunization	Type of reaction at the time of immunization	Date of bull virus injection	Results
100	Betal	9 December, 1944; goatis serum	Blocked reaction	23 March, 1945	No reaction
78	do.	do.	do.	do.	do.
91	do.	do.	Mild	do.	do.
70	do.	do.	Severe	do.	do.
76	Hill	24 November, 1944; Goatis serum	Doubtful	do.	Positive reaction
137	do.	do.	do.	do.	do.
155	do.	24 November, 1944; goatis alone	Mild	do.	No reaction
75	do.	24 November, 1944; goatis followed by serum 24 hours later	No reaction	do.	Positive reaction
3	Buffalo calves (controls)	..	....	..	Positive reaction (two died of rinderpest)

Field trial

Rinderpest was prevalent in the Kangra and Kulu valleys of the Punjab, which are areas of high rainfall and cool climate, because of the high altitude. There a stock of 600 Himalayan Gaddi goats was immunized by the serum-goatis method, 0·01 gm. of goatis and 15 c.c. serum; a few of the goats were not inoculated so that they might serve as controls. It was possible to keep regular temperature charts in the case of 10 animals only eight vaccinated and two controls. The controls died of the disease while the vaccinated ones survived the outbreak, thus confirming the results obtained at Hissar farm, namely that a combination of serum and goatis can be safely employed to produce immunity in goats against rinderpest.

(B) SHEEP

The experiments carried out on goats were repeated on Lohi, Hissari, Merino-Bikaneri crosses Bikaneri and *desi* sheep at the same time. Some variations in the reaction were observed in the different breeds. Goatis by itself was used as the immunizing agent in all breeds except the Lohi, in which because of the great susceptibility of sheep of that breed serum was used with the goatis.

Experiment No. 1

Three Lohi (497, 513, 712), five Hissari (149, 888, 893, 706, 915) and five Bikaneri (523, 511, 592, 591, 515) sheep were inoculated with 0·01 gm. of goatis; while two Lohis (No. 661, 521) were given a dose of serum along with goatis as a precautionary measure. The reaction in Lohi, except for the two which received serum and goatis mixture, was more marked than the one observed in other two breeds. In the Hissari sheep the reaction was milder than that of the Bikaneri and Lohi, four out of five animals reacted. Among the Bikaneri sheep the reaction was no different in those that got serum than in the others that did not get it. As the virus was attenuated, due to breakdown of the refrigerator, no definite conclusion could be arrived at. Most of the animals reacted on the sixth day and the reaction lasted for three or four days; in two animals it continued for about a week.

Experiment No. 2

As the goatis had been attenuated in the first experiment, six Bikaneri, two Lohi and two Hissari were given fresh goatis to observe the difference in the breed reaction if any. All the animals reacted on the fifth or sixth day. In all the animals the reaction subsided in a few days except in one in which it lasted for more than a week. None of these animals was dull. This showed that goatis could be used in these breeds as immunizing agents without any fear of severe reaction.

Experiment No. 3

Fifteen animals of Hissari, Lohi and Bikaneri breeds were inoculated with goatis to confirm the findings of the above experiment.

Hissari. Of these, eight reacted while seven (46·6 per cent) did not. The reaction was observed on the fourth day. In three animals the reaction lasted for one day (the day of reaction only), in three for two days and in two for more than three days. The duration of the reaction cannot be definitely stated, as the temperature was not taken up to the termination of the period because the animals were feeding normally. Only one was seen slightly dull.

One of the non-reactors (808), when being transported from the experimental yard to its pen, suddenly died on the way. On autopsy haemorrhages in the duodenum, ulcers in the intestine and an inflamed ileum were discovered. Parasitic nodules were also found in the intestine. On opening the buccal cavity, it was noticed that a clump of green bersseem which the animal had been chewing before commencing its journey was acting as an obstruction in the larynx; this probably led to aysphxia. The presence of enteritis and ulceration typical of rinderpest is rather interesting, because the animal was a non-reactor. It suggested that probably other animals had the disease but were naturally so resistant that they did not die or show any thermal reaction. This accident revealed something as to what was happening inside the body of the sheep.

Bikaneri. All the 15 animals reacted; 11 on the third day and four on the fourth day, but they retained their appetite.

No. of animals	Duration of reaction
3	One day
4	Two days
4	Three days
4	Four days

Lohi. These seem to be more susceptible as they showed a severe reaction. They were dull and off feed and seven of them had severe diarrhoea. Five of these were given serum and two left as controls, but all recovered at the same time, indicating that serum, given after the reaction had set in, was not of much use.

It may be mentioned here that the degree of dullness and partial loss of appetite did not, in many cases, correspond with the duration of the thermal reaction. This observation is similar to

what was observed in goats. In some animals, the thermal reaction lasted for a day but they remained dull for a long time. Dullness was usually observed two or three days after the thermal reaction when the animals stopped grazing. It was also found that goatis vaccinated sheep kept in better condition with grazing then with stall feeding during the period of observation.

Experiment No. 4

To confirm the above findings concerning the variation in the reaction of the different breeds of sheep, 10 more animals of each of the breeds mentioned above and eight *desi* sheep were inoculated with goatis. Lohi lambs were also included in the experiment to compare their susceptibility with that of the adults. For the sake of comparison, five of these were given serum goatis and five goatis alone.

Hissari. In this lot one animal did not react.

Five animals reacted on the third day,
Two animals reacted on the fourth day,
One animal reacted on the second day, and
One animal reacted on the first day, *i.e.*, one day after inoculation.

None of these animals stopped grazing for any length of time. The only sign of dullness was the fact that the animals did not feed on the day of reaction and a day after. The duration of the reaction was longer in this batch than in the previous one. One animal died with haemorrhages in the abomasum, enteritis, pneumonia of the anterior and middle lobes of both lungs and congestion of the larynx, thus indicating that the reaction in Hissari may not always be as mild as was indicated by the previous experiment.

Bikaneri. None of the animals had diarrhoea or were dull in spite of the longer duration of the thermal reaction as compared to its duration in the previous experiment.

One animal reacted on the first day,
Two animals reacted on the second day, and
Seven animals reacted on the third day.

Adult Lohi. Four sheep were injected with goatis while the other group of five got 15 c.c. serum and goatis. All were found to be more susceptible than the Bikáneris and Hissaris as observed previously. Most of the animals reacted on the second day and the reaction lasted for more than a week. In the animals which got serum, the reaction was comparatively mild, though it started in most of them on the third day and lasted for more than a week.

Lohi lambs. Two of these died without showing any lesions of rinderpest. The others reacted severely, demonstrating that the lambs were more susceptible than the adults. The dose of serum given did not seem to reduce the severity of the reaction, thus showing that a higher dose was required in young lambs.

Desi. These were found to be more resistant than the others because during the reaction they were quite active and fed normally. One died without showing any lesion of rinderpest. One did not react, while the remainder reacted on the second day, the reaction lasting from 3 to 10 days. The old animals exhibited the same degree of reaction as the young ones thus indicating that no immunity is conferred with age.

Field trial

At the time when goatis was being tried in goats at Kangra, it was also used in sheep. It was observed that Biangi sheep were comparatively more susceptible than the Gaddi and other mixed bred local sheep. Haddow [1939] on the other hand, has stated that sheep from Tibet and Himalayan hinterland are not susceptible. Of the Biangis, 150 were given goatis and serum while 44 were given goatis alone. None died but the thermal reaction was severe in the controls, while in the others the reaction was mild. Regular temperature charts were kept in the case of ten animals in each group; three of the ten animals which got goatis and serum (10 c.c.) did not react (probably examples of blocked reaction). The temperature record was maintained in ten Gaddi sheep also and individual

observations made. Out of these ten animals, two did not react. The day and the duration of reactions are as stated below :

Six reacted on the fourth day,
One reacted on the fifth day, and
One reacted on the sixth day.

One animal on the two days,
Two animals on the six days, and
One animal on the eight days.

There was a slight loss of condition amongst the reactors, but otherwise the results were satisfactory. In one lamb there were symptoms of haemoglobinuria. Blood smears were sent to the Punjab Veterinary College and piroplasma were found, but these could not be discovered in another smear sent to Hissar. From the results of this field trial it can be confidently assumed that goatis can safely be used in Gaddi as well as Biangi sheep for the purpose of producing active immunization against rinderpest. In the case of Biangi sheep if a high thermal reaction, which causes no mortality, is to be avoided, serum may be given in addition as in the case of goats.

IMMUNITY TEST IN SHEEP

The immunity of the sheep which were immunized with goatis was tested with bull virus. Seven Lohis, six Hissaris and 11 Bikaneris were used. Five Lohis and five Hissaris were kept as controls. Three buffalo calves used as controls in the case of goats automatically became controls in this experiment.

(*i*) *Lohi.* It was found that Lohis which had reacted to goatis did not react to bull virus after an interval of three months. Nos. 661 and 521 which had been given serum and had reacted mildly got a mild and passing reaction. One sheep died of pneumonia. The controls with the exception of one had high thermal reaction but did not exhibit dullness or loss of appetite. The severity of the reaction exhibited in the case of bull virus was less than in the case of goatis. The results are tabulated in Table VII.

TABLE VII

Results of immunity test in Lohi Sheep

Nos. of sheep	Date of immunization	Date of injection of bull virus	Results
669	24 November, 1944; positive reaction	23 March, 1945	No. reaction.
583	do.	do.	do.
497	30 October, 1944	do.	do.
712	do.	do.	do.
513	do.	do.	do.
661	30 October, 1944; serum goatis given, no reaction	do.	Rise of temperature on the 4th day
521	do.	do.	Rise of temperature on the 3rd day
611	Control		Positive reaction
548	do.		Reaction 4th day—7 days
556	do.		3rd day—7 days
524	do.		No reaction
506	do.		3rd day—4 days

Hissari. Out of six animals only one reacted, thus proving that goatis confers immunity to most of the animals and is solid up to three months. The causes or the reasons for the breakdown of immunity in goat No. 893 are not clear. The two of the controls did not react to bull virus and were probably naturally immune. Merinos are considered to be resistant to rinderpest, and as Hissari have Merino blood in them, resistance may partially be attributed to it. The reaction in

Hissaris was milder with bull virus than with goatis and in this respect they resembled the Lohis. The results are tabulated in Table VIII.

TABLE VIII

Results of immunity test in Hissari Sheep

Nos. of sheep	Date of immunization	Date of test	Result
915, 706, 149, 888 . .	30 October, 1944 ; Positive reaction	23rd March 1945 . . .	No reaction
893	do.	do.	Positive reaction
126	24 November, 1944 ; positive reaction.	do.	No reaction
835	Control		4th day—3 days
834	do.		No reaction ; it gave birth to a healthy lamb
844	do.		3rd day—3 days
612	do.		No reaction
676	do.		3rd day—5 days

Bikaneri. Out of 11 animals, seven did not show any reaction to bull virus but four did (35 per cent) thus showing that immunity produced is of a variable nature. The reaction observed in four was quite mild. The reaction in this breed is mild in case of goatis as well as bull virus.

All these experiments reveal that goatis and bull virus do not produce severe reaction in sheep. with the exception of those of the Lohi breed and that there are variations in type of reaction in each breed. Sheep as a whole are thus less susceptible than goats. DeCosta [1937] has made a similar observation in the case of sheep in Bareilly and Kumaun. It would appear that when the virus of rinderpest causes death among sheep in natural outbreaks, either it has assumed virulence through some natural factors such as climate or passage through the species or the virus of rinderpest in sheep is perhaps different from the virus of rinderpest in cattle. It has also been observed by various workers in the field that in some outbreaks of rinderpest all kinds of stock succumb to it, while in others cattle are only affected. As stated earlier in the Kangra outbreak, hill cattle as well as sheep and goats were affected, while in the outbreaks at Hissar and Ambala the disease was confined to sheep and goats.

EFFECT OF VARIATION IN DOSE

Sixty Hissari rams were to be sent to Kangra district for breeding purposes and as rinderpest was prevalent there it was decided that they should be immunized before despatch. As all these animals were available, different doses of goatis were given to observe the effect of dosage on the reaction produced. Rams were divided into six batches for this purpose as shown below :

First batch : *Dose per animal—0·01 gm. in 1 c.c. of saline*

Nos. of sheep	Day of reaction	Duration of reaction
475	3rd	4 days
337	,,	3 ,,
343	,,	3 ,,
308	No reaction	
501	6th	Day of reaction only
322	3rd	4 days
426	5th	3 ,,
380	3rd	4 ,,
306	3rd	3 ,,
706	4th	4 ,,
394	3rd	1 day
383	3rd	3 days
395	1st	3 ,,
429	3rd	2 ,,
	Average duration of reaction	$2\frac{6}{7}$

Second batch : *Dose per animal—0·02 gm. in 3 c.c. of saline*

Nos. of sheep	Day of reaction	Duration of reaction
421	No reaction	
253	3rd	3 days
248	3rd	7 ,,
370	3rd	3 ,,
317	3rd	7 ,,
372	3rd	3 ,,
344	3rd	3 ,,
334	3rd	6 ,,
295	3rd	4 ,,
313	4th	2 ,,
355	4th	3 ,,
348	4th	1 day
323	4th	3 days
	Average duration of reaction	$3\frac{1}{7}$,,

Third batch : *Dose per animal—0·005 gm. in 1 c.c. of saline*

No. of sheep	Day of reaction	Duration of reaction
320	No reaction	
271	do.	
319	4th	2 days
492	2nd	3 ,,
268	1st	2 ,,
391	4th	4 ,,
474	3rd	4 ,,
293	3rd	6 ,,
360	3rd	3 ,,
	Average duration of reaction	$3\frac{3}{9}$,,

Fourth batch : *Dose per animal—0·0025 gm. in 1 c.c. of saline*

Nos. of sheep	Day of reaction	Duration of reaction
382	4th	3 days
511	3rd	6 ,,
240	3rd	5 ,,
390	No reaction	
425	5th	1 day
311	4th	4 days
428	3rd	5 ,,
257	No reaction	
593	3rd	5 Stet
	Average duration of reaction	$2\frac{1}{9}$ days

Fifth batch : *Dose per animal—0·00125 gm. in 1 c.c. of saline*

Nos. of sheep	Day of reaction	Duration of reaction
488	4th	2 days
456	No reaction	
333	4th	5 ,,
274	4th	5 ,,
376	3rd	2 ,,
435	4th	4 ,,
345	No reaction	
368	Do.	
367	3rd	5 days
358	4th	1 day
	Average duration of reaction	$2\frac{1}{2}$ days

Sixth batch : Dose per animal—0·04 gm. in 1 c.c. of saline

No. of sheep	day of reaction	Duration of reaction
256	3rd	4 days
221	4th	3 ,,
316	3rd	5 ,,
407	4th	3 ,,
339	3rd	4 ,,
	Average duration of reaction	3¾ ,,

The thermal intensity of the reaction did not vary a great deal in each group though the duration of reaction was slightly different in each case. Cornell *et al.* [1941] stated that the size of the dose in bovines did not affect the course of the reaction in them. In this experiment, however, with a reduction of dosages there seems to be some reduction in the duration of the reaction, and an increase in the number of non-reactors. To some extent these variations may be accounted to individual factors based on the observations of the previous experiments, where so much variety of reactions was observed in different groups.

Summary

Anti-rinderpest serum (ordinary) and goat tissue vaccine given at the same time produced a solid immunity in Betal and hill goats against rinderpest, while the administration of the vaccine followed by serum 24 hours later caused a severe reaction. The above method has been safely utilized in the field and it was found that the dose of serum required in hill goats was slightly higher than that for Betal goats.

Goat tissue vaccine alone can be used to produce immunity against rinderpest in Bikaneri, Hissari (Merino and Bikaneri crosses) and *desi* sheep, but the use of serum is necessary in the case of sheep of the Lohi and Biangi breeds which are more susceptible. There are definite indications that all these breeds react differently to inoculation of the vaccine. Lohis are the most susceptible and react more or less like goats ; Lohi lambs react still more severely.

3. The reaction produced in sheep and goats by bull virus was milder than that of the goat tissue virus obtained from the Punjab Veterinary College. When different doses of goatis were given to sheep, no difference in the intensity of the reaction was noticed, though the duration of the reaction varied with the dose.

Acknowledgments

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AN ESTIMATE OF THE FOOD REQUIREMENTS OF MILCH AND OTHER CATTLE IN INDIA AND SUGGESTIONS AS TO HOW PRESENT AGRICULTURAL PRACTICE CAN BE ADJUSTED TO PROVIDE THEM*

By GRAHAME WILLIAMSON, Animal Husbandry Commissioner with the Government of India helped by K. P. R. KARTHA AND RAM SWARUP, Animal Husbandry Bureau, Imperial Council of Agricultural Research

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THE available statistics concerning cultivated crops, although they are admittedly inaccurate, do provide a reasonable working basis upon which to make an estimate of the fodder derived from these crops but in computing the part which the uncultivated sources of annual food play and which, after all, is the real mainstay of the live-stock of the country, only a few vague indications may be gathered from published figures. Previous authors have used these in different ways and it is only a matter of opinion as to how close or how wide they have come to actuals.

The figures arrived at in this note, like those previously computed, are not absolute but can be taken only as an approximate indication of the most probable requirements and deficiencies. Only British India has been considered.

The following cattle population has to be fed :—

	Cattle	Buffaloes	Total
	(Millions)	(Millions)	
(*a*) Weaned young stock (1 to 3 years old)	17·4	5·9	23·3
(*b*) Idle male stock	2·2	0·2	2·4
(*c*) Cows	36·4	15·0	51·4
(*d*) Bullocks and bulls	44·6	4·7	49·3

The major sources of cattle food are : (1) grass, (2) specially grown fodder crops, (3) straws of food grains, (4) cotton seed and oilcakes, (5) by-products such as bran.

Sufficient grass grows in the country each year to provide an immense quantity of food for animals but, in practice, most of it has to be obtained by grazing and thus its usefulness is limited to certain classes of live-stock, that is, those which have time to graze and need no special form of concentrates. It is considered that these resources are available for and can meet the requirements of idle male stock and all cows (excluding female buffaloes used for milk production), that they meet half the maintenance requirements of working bullocks, as these animals are fully employed for about half the year only, and that growing young stock can obtain all their requirements from this source. The amount of milk produced to-day in British India is estimated to be 19·7 million tons, of which 3·3 million tons are used by calves. It is desired to increase that output some three times but that target is, for the time being, unattainable and all that seems practicable is an increase of 50 per cent to bring the total to 28 million tons. This involves no increase in the number of cows.

For purpose of calculation here, the food requirement other than those supplied by grass can most conveniently be expressed in terms of starch equivalent (S. E.) and digestible protein (D. P.) and the amounts of these which are needed are given in Table I.

* Presented before the Crops and Soils Wing Meeting held in December 1945 at Delhi.

TABLE I

The starch equivalent and digestible protein required

	Total requirements in million tons per annum	
	S.E.	D.P.
Production requirements for 28 million tons of milk	8·85	1·43
Maintenance of 14 million buffaloes (average live weight 1,000 lb.)	13·80	1·38
Maintenance of 49·3 million working bullocks and bulls (average weight 600 lb.) for six months	19·79	1·64
Requirements for work (medium work 4 hours daily at 4·1 lb. S.E. and 0·8 lb. D.P. per day)	33·32	6·56
Total	70·76	11·01

The ratio of D. P. to S. E. required is 1 : 6·43. The nature of the foods which could provide that, the amounts available and the extent of the deficiency is stated in Table II.

TABLE II

Food necessary to provide the required proportion of D. P. and S. E.

	Proportions required	Percentage of composition		Present production in million tons	Required in million tons	Deficiency in million tons
		S.E.	D.P.			
Green fodder	20	10	1·5	78·1	182·6	104·5
Straw	12	22·5	0·0	87·2	109·6	22·4
Cakes and seed	3	73·0	25·0	3·4	27·4	24·0
Rice and wheat bran	2	43·0	8·0	1·3	18·3	17·0

The gap between requirements and present production is very wide. In trying to plan how these quantities can be made good, the human requirements must, of course, receive primary consideration but by a readjustment of the cropping programme it appears possible to meet the needs of both human beings and cattle.

The cultivated land in India is now divided for crops roughly as under :

	Million acres
Food grains excluding gram	174
Gram	15·5
Oilseeds	15·5
Cotton seed	14·0
Fodder crops	10·5
Others	50
Total	280·5
Cultivable waste	92
Forests	67
Not available for cultivation	94
Total	533·4

The present production of cereals is estimated by the Nutrition Advisory Committee of the Indian Research Fund Association to be 13 per cent short of human requirements and there is a 20 per cent shortage of pulses and a 330 per cent shortage of oils and fats. It has been accepted that as a result of various measures under consideration of the Governments, it is not unreasonable to assume that

there will be a 50 per cent increase in the yield from land in 10 to 15 years time. As that target is attained it will be possible to produce 13 per cent increase in the present quantity of cereals on 131 acres, releasing 43 million acres which may be devoted, as suggested below, towards making up deficiencies in human and livestock requirements.

As stated above the production of oils and fats needs to be increased 330 per cent in order to provide food for human beings. At the increased rate of yield, the 15·5 million acres now used must be increased to 34 million acres and thus 18·5 million acres more have to be used for that crop. At present 2·5 million tons of edible oil cakes are produced. When the area and yield are increased, 8·25 million tons will be available. Adding the uncrushed cotton seed which is fed at present, that is 0·94 million tons, the total of cakes and seed will amount to 9·2 million tons. No increase in the acreage under cotton seed is envisaged.

To augment the supplies of concentrated food it is suggested that the present acreage under gram and barley, which are good cattle feeds, may be increased as much as possible or crops of equivalent value where gram and barley cannot be grown. Six million acres are, however, required for the cultivation of green fodder ; the remaining 18·5 million acres can be devoted to the cultivation of gram and cereals for cattle. On that acreage, 4·5 and 5·32 million tons respectively can be raised.

The six million acres which may be allotted to green fodder crops along with the 10·5 million acres at present used should provide, on the increased yield scale, the amount required. More nutrients can be made avilable from this land if leguminous fodder like lucerne and berseem are grown but, assuming that the present proportion between the various fodders persists, the 50 per cent increase of the present out put of 7·5 tons per acre on 16·5 million acres will produce 185·6 million tons at 11·25 tons per acre. Straw will remain practically the same as at present. The food provided when the above change is introduced in the cropping programme, will be as indicated in Table III.

TABLE III

Food provided after changes in the cropping programme

Feeding stuffs	Total weight		S.E.		D.P.	
	Required in million tons	Feed provided for in millions tons	Required	Provided for	Required	Provided for
Green fodder	182·6	185·6	18·26	18·56	2·74	2·78
Straw	109·6	114·4	24·66	25·74	..	..
Total			42·92	44·30	2·74	2·78
Cakes and seed	27·4	9·15	20·00	6·68	6·85	2·29
Rice and wheat bran	18·3	1·47	7·87	0·63	1·46	0·12
Gram		4·5	..	3·20	..	0·59
Barley		5·32	..	4·04	..	0·39
Total			27·87	14·55	8·31	3·39
Grand Total			70·79	58·85	11·05	6·17
Shortage				11·94		4·88

The only way in which this deficiency can be made up is by an extended programme of making hay or silage out of the surplus grass which is available during monsoon, and which is otherwise wasted. There are 136 million acres of grazing land in British India. No precise data are available as to how much grass this land produces. The limited information we have goes to show that the production of grass is about two tons green per acre. A third of this can be made into hay or silage,

giving the equivalent of 90 million tons of green grass. Assuming the grass to have the value of 6·2 per cent S. E. containing 1·33 D. P., it will provide 5·6 million tons of S. E. with 1·2 million tons of D. P. This still leaves a deficiency of 6·34 million tons of S. E. and 3·68 million tons of D. P. That can be met from concentrated protein food of animal origin of which fish meal should supply a large proportion.

It is recognised that while the ultimate objective must be the provision of food which will allow the cattle of this country to maintain health and to produce on the most paying basis, it is necessary in practice to concentrate on intermediate goals which can be progressively raised as agricultural conditions improve. These intermediate goals must differ from region to region according to markets, agricultural practices, prejudices, etc.; the exact goals are, therefore, a matter of individual administrations to determine.

It is improbable that administrative units can become self-sufficient; inter-adjustment in the supply of protein concentrates must continue. That class of foodstuffs being relatively non-bulky is transportable and thus the difficulty is not insoluble. Table IV illustrates the present position in regard to them.

TABLE IV

Present position in regard to foodstuffs

Province	Present food available expressed as		Ratio of D.P. to S.E.
	S.E.	D.P.	
Punjab	6,206,579	673,703	1 : 9·21
Sind	623,817	49,877	1 : 12·5
Bengal	2,933,233	87,165	1 : 33·66
Assam	626,548	17,789	1 : 35·22

The foregoing estimates give what is considered to be an all-India picture of the stock fee position and needs. As a first stage towards the ultimate aim, attention should be given to the production of what is now recognised to be the primary human food requirement from cattle as expressed by the Nutrition Advisory Committee of the Indian Research Fund Association (1944) and the Agricultural Policy Committee No. 5 (Agriculture, Forestry and Fisheries), that is, the production of more milk and the work entailed in cultivating the land.

As far as the latter need is concerned, it must be admitted that what bullocks there are do in fact cultivate the present acreage although on their low nutrient level they do not do so in an economical manner but, for the immediate present, the standard as it is must be accepted.

Such, however, need not be the case in regard to increased milk production for it has been abundantly proved that immediate returns are to be had from better feeding. If these returns are to be maintained and increased, attention must be paid to the development of the rising and future generations of cows. As an immediate object then, the requirements may be expressed as the production of food for 8·1 million tons of milk per annum, *i.e.*, 50 per cent more than the present supply and for the growth of heifers. The food required is extra to that now grown and it must be produced largely from concentrates because (1) the maintenance requirements of the cows and buffaloes and the present supply of milk is mainly derived from the bulky uncultivated food; (2) a milk production ration necessitates a high proportion of proteins; and (3) the milk is required for heavily populated areas and if it is to be delivered there in the fluid state, it must be produced in or near these areas; such areas have few grazing facilities and so, for the most part, the milch animals and special young stock must be stall fed. A certain amount of green fodder must be provided because there is a lack of it in the present ration. The requirements, as far as milk is concerned, may, therefore, be stated as 2·56 million tons of S. E. and 0·47 million tons of D. P. with a nutritive ratio of 1 : 6.

The following feeding stuffs (Table V) fed approximately in the proportion stated below, will provide that :

TABLE V

Extra feed required for milk

	Average per cent of S.E.	Average per cent of D.P.	Proportion	Quantity recorded in million tons
Green fodder	10	1·5	20	10·22
Cakes and oil seeds	73	25·0	1	1·51
Gram or other legumes	71	13·0	2	1·02
Cereal by-products	43	8·0	2	1·02

As regards feed for heifer calves, it is estimated that 8·8 million of the present female young stock are undergrown and that early returns may be expected from better feeding. The nature of the concentrates they need may be expressed as indicated in Table VI.

TABLE VI

Extra feed for heifer calves

	Proportion	Quantity (in million tons)
Cakes and oil seeds	2	0·32
Gram or other legumes	1	0·16
Cereal by-products	2	0·32

The total extra requirements for both forms of production and the present estimated production are given in Table VII.

TABLE VII

Total extra requirements

	Estimated present production	Extra quantity required	Percentage of increase desired
	Million tons	Million tons	
Green fodder	78·1	10·22	13
Cakes and oil seeds	3·4	0·83	25
Gram and pulses	..	1·18	100
Rice and wheat bran	1·32	1·34	102

A plan to meet these needs may be formulated on the same basis as that previously used but in this case the proportions of the foods are somewhat different and although it cannot be assumed that a 50 per cent increase in the land yield can be expected in the time under consideration, calculations may be based upon a proportionate increase of 25 per cent but again human requirements from the

land are of primary consideration. After human needs in cereals are met, 17 million acres of land will be released from these crops and simultaneously the production of cereal by-products will be increased by 0·13 million tons. By the same practice, 0·62 million acres will be released from pulse production. On the other hand, 12·4 million extra acres will be needed for oil crops if the target is to provide for even half of the present human deficiency, but it will provide 3·1 million tons of extra oilcakes. The immediate need of 10·22 million tons of green fodder should be covered in a 25 per cent increase in yield of the present acreage. The position then would be, in relation to the limited target set, that there is a surplus of 2·27 million tons of oilcake and a deficiency of 1·18 million tons of gram and pulses and 1·21 million tons of cereal by-products, while there is 5·22 million acres of land available for further use. It should be employed for the production of either gram, pulses or cereals which will approximately meet the balance of requirements.

AN ESTIMATE OF THE FOOD REQUIREMENTS OF MILCH AND OTHER CATTLE IN INDIA*

By L. C. SIKKA, Assistant Director of Agriculture (Livestock), Bengal

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THE Advisory Board of the Imperial Council of Agricultural Research has issued a plan for the development of Indian Agriculture and Animal Husbandry. In order to achieve national sufficiency in human foodstuffs (for a population of 400 millions), in that plan, the minimum targets laid down are to increase the existing production of cereals by 10 per cent, pulses by 20 per cent, fats and oils by 250 per cent, fruits by 50 per cent, vegetables by 100 per cent, milk by 300 per cent and fish and eggs by 300 per cent. Furthermore, with a view to provide adequate supplies of animal foods necessary for the increased outturn of work and milk, it has been stated that the present production of oil cakes and other concentrates (estimated at 3·75 million tons) must be increased by 400 per cent and of roughages (estimated production 175 million tons) by 55 per cent. This note has been written (*a*) to show the approximate requirements of our bovine population in the light of that plan, and (*b*) to broadly indicate the adjustments, which may be made in the existing agricultural practice, in order to provide these requirements.

FOOD REQUIREMENTS OF THE INDIAN BOVINE STOCK

Estimates have to be formulated of requirements for (*a*) milk production, (*b*) work, and (*c*) growth of the bovine stock required to attain the foregoing targets. To be accurate such estimates must be based on the actual requirements of different nutrients (digestible protein, starch equivalent, etc.) for each purpose, a point overlooked, in most cases, in the past [Burns, 1944].

It should be obvious that because of the great variations in the quality and, therefore, requirements of animals in different parts of India, the all-India estimate of requirements ought to be a composite of regional estimates. Unfortunately, however, it is not possible to prepare regional estimates with any measure of accuracy. This is because, firstly, it is not easy to judge correctly the number of work and milch animals and youngstock that will be required in each region to supply its requirements, since that must depend upon the improvement which may be effected through better feeding, improved management, proper breeding, and disease control in the existing stocks of each region; and secondly our knowledge regarding the optimum food requirements of different types of cattle of each region is extremely imperfect. It is essential that immediate steps be taken to obtain this information, so that the regional estimates can be prepared accurately. Without that no definite planning can be possible. An all-India estimate, based on average estimated requirements for all regions, must, therefore, be of somewhat academic interest. However, it should serve as a first approximation in indicating the total requirements for the country, and thereby showing the lee-way we will have to make up to provide these requirements. It is, therefore, given here for what it is worth. Because of the paucity of the required data only an estimate for the British India is presented in this note.

The total quantity of milk required in British India may be taken at 44 million tons. Assuming that the existing annual production per animal is 750 lb., which may be increased by 60 per cent [Burns, 1944] through improved feeding, management and disease control (thus giving an annual yield of 1200 lb. per animal), some 82·3 million milch cattle will be required to produce the required quantity of milk. Taking into account the milk production by goats, the foregoing number of milch cattle may be reduced to 81 millions, compared with the existing number of 54 millions (*Agricultural*

* Presented before the Crops and Soils Wing meeting held on December 1945, at Delhi.

Statistics of India, 1935-36). Assuming the average live weight per head to be 600 lb. the nutrients required for these animals willl be :

	Digestible protein in tons	Starch equivalent in tons.
For maintenance (0·5 lb. digestible protein and 5·0 lb. starch equivalent per 1,000 lb. live-weight)	3,959,600	39,596,000
For production (0·6 lb. digestible protein and 3·0 lb. starch equivalent per gallon, .	2,644,000	13,200,000
Total .	6,603,600	52,816,000

It is significant to point out that the foregoing requirements for milk production can be reduced if the average milk production per animal can be improved by more than 60 per cent. This is because, in that case, a smaller number of animals will be required to produce the total quantity of milk required, which, in turn, will lead to a reduction in the nutrients required for their maintenance. This should indicate the great need of improving the production of our stock by breeding since the scope of improvement by better feeding and management, although large at present, is strictly limited by their hereditary make-up.

According to the *Agricultural Statistics of India*, 1935-36, the total number of bulls and bullocks in British India is 57·2 millions. Ware [1944] *Ibid* has estimated that the number of existing bullocks can be reduced by 15·2 millions as a result of improving their efficiency by 60 per cent (in the more backward areas only) through better feeding, management and disease control. This, if accepted, would leave 42 million bulls and bullocks to be fed. Taking their average live-weight at 600 lb. per head, their production requirements at 0·5 lb. digestible protein and 2·5 lb. starch equivalent per day, and the average working period at 4 months per annum their requirements will be :

	Digestible protein in tons	Starch equivalent in tons
For maintenance	2,053,100	20,531,000
For work	1,140,600	5,703,000
Total .	3,193,700	26,234,000

According to the *Agricultural Statistics of India*, 1935-36, the total number of youngstock, of all ages, in British India is 48 millions. We need all of them to give us the number of milch and draught animals we require. Assuming their average live-weight to be 350 lb. per head, and the nutrients required for growth to be 0·4 lb. digestible protein and 2·0 lb. starch equivalent per day, their requirements will be :

	Digestible protein in tons	Starch equivalent in tons
For maintenance	1,368,700	13,687,000
For growth	3,128,600	15,643,000
Total .	4,497,300	29,330,000

By addition of the foregoing quantities of nutrients required for milk production, works, and growth, the following estimate of the total requirements of our bovine stock is obtained.

Digestible protein	14,294,600 tons.
Starch equivalent	108,380,000 tons.

AVAILABLE SUPPLY OF ANIMAL FOOD

Crop residues (straw and stover of cereals and pulses), grasses and weeds, grazed or collected from grazing lands, waste lands and cultivated lands, specially grown fodder crops, by-products of the oil and milling industries, and a certain amount of seeds, comprise the food of our cattle. It is not possible to formulate an exact estimate of the supplies of each, as the basic data required for this purpose is wanting. Any attempt in this direction must, therefore, naturally be in the nature of a guess estimate. This must be borne in mind in considering the figures which follow.

Ware [1944] has estimated that the existing supply of animal food consists of 19·53 million tons of special fodder crops, 67·74 million tons of grasses, 87·2 million tons of straws of food grains, and 3·729 million tons of concentrates (cakes, seeds, bran and pollard). Details have, however, not been furnished how these estimates were arrived at. It would appear, the estimate for concentrates is rather low, provided all the available supplies of oil-cakes are made available for the feeding of livestock, which, of course, is not so at present, as substantial quantities are used as manure. Burn-[1944] has estimated the production of oil-cakes alone to be three million tons. When to this are added the quantities of cotton seed, wheat bran, rice bran and pulse husks which can be available for the feeding of cattle, the total quantity of concentrates must considerably exceed Ware's estimate.

Our estimate of the total quantity of animal food produced (not the quantity that is actually fed to cattle) in British India, and the nutrients supplied by it, is as follows :

	Total production (dry weight) in Million tons	Digestible protein in tons	Starch equivalent in tons
Roughages :			
Fodder crops	19·53	976,000	7,812,000
Grasses	67·74	3,387,000	27,096,000
Straws	87·20	436,000	21,800,000
Total	174·47	4,799,000	56,708,000
Concentrates :			
Oil cakes (excluding castor)	3·00	961,000	2,076,000
Brans	1·40	122,000	714,000
Pulse husks and *churi*	1·37	65,000	709,000
Cotton seed	1·40	157,500	1,078,000
Total	7·17	1,305,500	4,577,000
Grand Total	181·64	6,104,500	61,285,000

Compared with the total requirements, the available supplies of digestible protein and starch equivalent are 42·7 per cent and 56·5 per cent respectively.

ADJUSTMENTS RECOMMENDED TO MEET THE DEFICIT

It should be obvious that there cannot be a uniform set of adjustments for the whole of India. These must vary from area to area in accordance with the requirements of each, and its agricultural conditions. Furthermore, it is unlikely that each area will prove capable of being made self sufficient, although like the deficit areas, there are bound to be surplus areas as well. The surplus produce of the latter should be available for supplementing the produce of the former.

For want of the requisite information, it is not possible to treat the subject on a regional basis. Only all-India adjustments are, therefore, indicated in what follows. But steps must be taken to secure the required information for each region and sub-region, and detailed plans prepared on the basis of such information to secure a regional self-sufficiency as far as possible.

It is well known that the same land cropped with cereals and pulses can support a much larger population than if devoted to stock keeping. Agriculturally over-populated as India is, with its small

holdings, the land must needs be cropped essentially to produce human food of plant origin. The production of animal food must remain largely subservient to that system, only surplus land being devoted to the keeping of livestock, that in the absence of land, must subsist on the by-products of human food.

As already stated, in order to attain self-sufficiency in human foodstuffs, the targets fixed are to increase the production of cereals by 10 per cent, of pulses by 20 per cent and of fats and oils (and, therefore, of oil seeds) by 250 per cent. That, when done, should also enhance the existing supplies of straw by some 15 per cent, bran by 10 per cent, pulse husks and *chuni* by 20 per cent, and oil-cakes by 250 per cent, thus giving us the following quantities of nutrients :

	Total production (dry weight in million tons)	Digestible protein in tons	Starch equivalent in tons
Roughages :			
Fodder crops	19·53	976,000	7,812,000
Grasses	67·74	3,387,000	27,096,000
Straws	100·28	501,400	25,070,000
Total	187·55	4,864,400	59,978,000
Concentrates :			
Oilcakes (excluding castor)	7·50	2,402,500	5,190,000
Brans	1·84	134,200	785,400
Pulse, husks and *chuni*	1·55	78,000	850,800
Cotton seed	1·40	157,500	1,078,000
Total	11·99	2,772,200	7,904,200
Grand Total	199·54	7,336,600	67,882,200

Compared with the total requirements, the supplies of digestible protein and starch equivalent, which will thus become available, are 53·4 per cent and 62·6 per cent respectively.

It must first be considered how this projected increased production of human food (and, therefore, of animal food) may be effected : whether by extending the area under these crops, or by obtaining higher yields per acre, or by a combination of both these steps.

Burns [1944]. *Ibid* has already indicated the technological possibilities of crop improvement in India. He has shown that by adopting improved agricultural practices it is possible to improve the yield of cereals by 20-50 per cent, of grain by 20 per cent, and of most oilseeds by 15-25 per cent. In view of these results, the desired increased production of cereals and pulses should be obtainable from the existing areas under such crops ; while in case of oilseeds their present area (18·5 million acres) must be at least doubled. However, in view of the great possibility for increasing the yield of cereals, and their large area (156 million acres), there should be ample scope for finding the additional land required for growing oilseeds out of that area.

It should also be stated that the use of edible oil-cakes as manures must be discontinued. Burns [1944] *Ibid* has estimated that, in order to meet only one-fifth the manurial needs of paddy (to secure increased yield per acre), the whole of the existing supply of oilcakes is required. If the use of edible oilcakes as manure is to increase, the animal food supply position may further deteriorate. No doubt, without the use of manures the scope for increasing the yield of our crops is, in most cases, very little ; but artificial fertilizers, farm yard manure, compost and green manures, rather than edible oilcakes, must be looked upon to satisfy that need.

It is also essential to stop the export of oilseeds and oilcakes, and develop the Indian oilseeds-crushing industry.

In spite of the foregoing increased production, there will still remain a deficit of some 6·66 million tons of digestible protein and 40·50 million tons of starch equivalent. How may that be met ?

Obviously, the first thing to do would be to increase the supplies from the existing sources, as much as possible, and then look for new sources. The waste lands, aggregating some 90 million acres in British India, offer a great scope in this respect. These should be surveyed, and working plans formulated for their development. Such waste lands, as can be brought under cultivation economically should be used for that purpose, if possible, for growing fodder crops ; while the grass production from the rest should be increased by suitable management. That there is a very great scope in the latter direction, there can be no doubt. We have already the example of improvement of usar lands in the United Provinces*, the hay production of which was increased from 2·75 maunds to 15·0 maunds per acre over a period of $5\frac{1}{2}$ years by the simple and inexpensive practice of protecting them from grazing during the six rainy months, in spite of grazing having been permitted after the hay harvest. If each acre of waste land, suitably managed, were to yield annually even half a ton (dry weight) of hay more than at present (surely, not an impossible target), the extra quantity of grazing material thus obtained would provide nearly $2\frac{1}{4}$ million tons of digestible protein and 18 million tons of starch equivalent.

In order to produce the balance—approximately $4\frac{1}{2}$ million tons of digestible protein and $22\frac{1}{2}$ million tons of starch equivalent—we must again look to our cultivated land. The total cultivated area of British India approximates 260 million acres, of which some 45 million acres are classified as ' current fallows '. Only about 30 million acres are double—cropped at present, the percentage of double-cropped area being nearly 14 per cent of the net-sown area. The required quantity of animal food should be obtained through extending the double-cropped area by raising a catch crop of green fodder, either before or after the principal crop as feasible. Provided the catch crop is a legume there should be no danger of depleting the soil fertility by this practice. The raising of a crop of pulse from paddy land, by broad-casting the pulse seed (*khesari*) in the standing crop of paddy†, is an admirable practice, which must be extended with advantage both to the main crop and cattle. Similarly suitable pulses can as well be grown on lands producing other cereals. Where means of cheap irrigation are available, the extension of area under berseem and other clovers should be possible. To increase the area under leguminous fodders by 30 million acres should, therefore, not prove impracticable.

SUMMARY

To summarise, the following are the adjustments recommended in the existing cropping with a view to produce the required quantity of animal food :

Crop	Area (million acres) under	
	Existing cropping	Proposed cropping
Cereals	156·0	137·5
Pulses (for grain)	27·0	27·0
Oilseeds	18·5	37·0
Fodder crops	10·5	40·5
Other crops	32·0	32·0
Total	244·0	273·5

REFERENCES

Burns, W. [1944]. Technological Possibilities of Agricultural Development in India.

Ware, F. [1944]. *Ibid.*

**Proceedings of the 2nd meeting of the Animal Husbandry Wing held in Madras, 1936, p. 244.*

†*Proceedings of the Cattle Conference* held at Simla, 1937, p. 59.

A SURVEY OF THE FEEDS AND FODDERS AND THE REQUIREMENTS OF THE LIVESTOCK IN THE UNITED PROVINCES*

By N. C. Das Gupta, Research Officer, Cattle Feeding Research Station, Bharari, Jhansi

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THE products of the field which are inedible for man are the main source of nutrients for the livestock but, farm animals cannot produce milk, meat or labour efficiently unless some edible food is also included in the ration. In addition to the mill by-products, a large amount of grains are required for this purpose. Hence, to study the position of feeds for the livestock of a country it is necessary to find out the amount of food which can be spared after meeting the requirements of the human population.

Food requirements for human population

According to the census report of 1941 there are 29,542,474 male and 26,803,981 female in the United Provinces and the approximate amount of nutrients necessary per year is shown in Table I.

Table I

Requirement of nutrients for human population per year

Population		Protein		Fuel	
		Per adult per day	Total per year in thousand tons	Therms per adult per day	Total therms in thousands
Male	29,542,475	65 gm	689·8	2·600	28,030,000
Female	26,803,981	55 gm	529·5	2·080	20,340,000
Total	56,346,456	..	1,219·3	..	48,370,000

The figures are on the basis of nutrients required by the adults [*Health Bulletin*, 1937].

For a mixed population of men, women and children the requirement is expected to be lower but, as the extra nutrients required for heavy work, pregnancy and nursing have not been taken into account, it is advisable to assume the figures arrived at as what is required to support the total population of the province.

Nutrients available for human consumption. The acreage under different crops [*Season and Crop Report of the United Provinces for the year 1942-43*] and their present estimated yield [Pugh and Dutt, 1940] have been taken into account to arrive at the total food produced. In some cases the figures had to be assumed in the absence of statistical averages. For example, nutrients of the item 'other food crops' have been calculated from the average figure of the pulses. Similarly, the figure for the fruits and vegetables is based on the average of all the common vegetables. Thus they provide only an approximate return. *Health Bulletin* No. 23 has been followed for the food values.

*Presented before th e Crop and Soils Wing Meeting held in December, 1945, at Delhi.

TABLE II

Nutrients available for human consumption

Crop	Area in thousand acres	Yield per acre (lb.)	Total yield in thousand tons	Protein		Fuel value	
				Per cent	Total in thousand tons	Therms per ton	Total therms in thousands
Rice	6,902	800	2,465	7·57	186·6	3544	8,736,000
Wheat	7,397	900	2,972	11·77	349·7	3510	10,420,000
Barley	4,130	857	1,580	11·40	180·1	3401	5,373,000
Jowar	2,590	428	495	10·42	51·5	3584	1,773,000
Bajra	3,040	407	553	11·59	64·1	3657	2,022,000
Mandua and sawan	811	300	108	7·70	8·4	3333	361,800
Kodon	1,307	300	175	8·31	14·5	3132	548,100
Maize	2,424	842	909	12·91	117·3	3640	3,309,000
Gram	6,080	568	1,541	17·08	263·1	3664	5,645,000
Other food crops	4,715	400	842	23·00	193·6	3454	2,909,000
Potato	138	16,400	1,009	1·73	17·5	1009	1,017,000
Fruits and vegetables	471	16,400	3,449	1·35	46·6	417	1,444,000
Sugarcane	1,865	3,250 (*gur*)	2,706	0·36	9·7	3883	10,510,000
Til oil	264	90	11	..	..	9143	97,090
Mustard oil	185	192	16	..	..	9143	145,200
Groundnut oil	116	108	6	..	..	9143	51,360
Milk-cow	..	..	1,410	3·18	44·8	839	1,183,000
Milk-buffalo	..	..	1,637	3·80	62·2	1075	1,760,000
Total	42,435	..	21,884	..	1,619·7	..	57,304,550
Requirements for human consumption.					1,219·3		48,370,000
Balance					400·4		8,934,550

Meat and poultry also provide nutrients for human consumption, but for reasons discussed elsewhere they have not been considered. After providing for the human population it is found that 400·4 thousand tons of protein and 8,934,550 thousand therms of fuel can be spared for use of livestock.

NUTRIENTS REQUIRED FOR LIVESTOCK

The data of the livestock population collected in 1944 [*Return of Live-stock and Agricultural Machinery and Implements for 1944*] have been utilized for arriving at their requirement of nutrients. The minimum standard of feeding recommended by Morrison [Morison] has been followed for this purpose.

TABLE III

Requirement of nutrients for livestock per year

Particulars	Number	Average Live-weight (lb.)	Dry matter		Digestible proteins		Net energy	
			Per 100 lb. live weight per day (lb.)	Total per year in thousand tons	Per head per day (lb.)	Total per year in thousand tons	Therms per head per day	Total Therms in thousand
Cattle and buffaloes								
(a) *Maintenance*								
Cows above 3 years in milk	2,303,083	700	2·7	7,094	0·44	165·1	4·36	3,665,000
Cows above 3 years dry	2,631,107	700	2·4	7,204	0·44	188·7	4·36	4,188,000
Cows above 3 years not calved	390,896	700	2·4	1,070	0·44	28·0	4·36	622,200
Buffalo cows above 3 years in milk	2,130,258	850	2·7	8,000	0·52	181·6	5·16	4,028,000
Buffalo cows above 3 years dry	1,921,760	850	2·4	6,389	0·52	163·1	5·16	3,619,000
Buffalo cows above 3 years not calved	335,437	850	2·4	1,150	0·52	28·5	5·16	631,600
Total for milch animal	9,721,541			30,907		755·0		16,753,800
Cows above 3 years for work	3,278	700	2·4	9	0·44	0·2	4·36	5,217
Bullocks or bulls for work	10,079,142	800	2·2	28,910	0·49	811·4	4·90	18,039,000
Bullocks or bulls not in use for work or breeding.	136,086	800	2·4	426	0·49	11·0	4·90	243,400
Buffalo cows for work	11,477	850	2·4	38	0·52	1·0	5·16	21,620
Buffalo bullocks or bulls for work	809,598	1000	2·2	2,902	0·60	99·7	5·95	1,758,000
Buffalo bullocks or bulls not in use for work or breeding	13,659	1000	2·4	53	0·60	1·3	5·95	29,660
Cows above 3 years not in use for work or breeding	15,148	700	2·4	41	0·44	1·1	4·36	24,110
Buffalo cows not in use for work or breeding	10,146	850	2·4	34	0·52	0·9	5·16	19,110
Total for maintenance	20,800,075			63,320		1,681·5		36,884,917
(b) *Production*								
Milk								
Cow	1,410,000 (tons)		fat 5 per cent			64·9		1,036,000
Buffalo	1,637,000 (tons)		fat 7 per cent			93·3		1,518,000
Total for milk						158·2		2,554,000
Work								
Bullocks, bulls and cows on the basis of medium work						657·2		14,320,000
Buffalo bullocks, bulls and cows on the basis of medium work						53·5		1,169,350
Total for work						710·7		15,489,350
Total for production						869·0		18,043,350

TABLE III—*contd.*

Requirement of nutrients for livestock per year

Particulars	Number	Average live-weight (lb.)	Dry matter		Digestible protein		Net energy	
			Per 100 lb. live-weight per day (lb.)	Total per year in thousand tons	Per head per day (lb.)	Total per year in thousand tons	Therms per head per day	Total therms per thousands
(c) *Growth, including maintenance* Calves 0-1 year								
Cattle	2,464,743	125	2·2	1,105	0·33	130·5	1·75	1,574,000
Buffalo	1,584,830	150	2·2	853	0·41	105·9	2·30	1,330,000
Young stock 1-3 years—Cattle	3,059,276	400	2·4	4,786	0·80	398·8	5·50	6,128,000
Buffalo	1,685,855	500	2·4	3,298	0·87	239·1	6·10	3,754,000
(d) *Breeding*								
Bulls	15,481	900	2·2	50	0·70	1·8	7·25	40,970
Buffalo bulls	11,223	1200	2·2	48	0·84	1·5	9·05	37,060
Total for growth and breeding	8,821,408			10,140		877·6		12,864,030
Total for cattle and buffaloes	20,621,483			73,460		3,428·1		67,792,297
Horses and mules above one year for medium work including maintenance	364,818	500	2·0	595	0·76	45·2	7·28	994,100
Donkeys for light work including maintenance	221,946	300	2·2	230	0·45	16·3	4·23	342,600
Total requirement for livestock	30,208,247			74,294		3,489·6		69,128,997

The yield of milk per year has been estimated on the basis of 800 lb. per lactation from a cow and 1,000 lb. from a buffalo [Wright, 1937]. The average period of lactation has been taken as seven months, and the annual yield has been obtained on the assumption that the number of milch animals remain constant throughout the year.

Sheep and goat subsist partly on shrubs and bushes and partly on the common feeding stuffs. The shrubs and bushes are not usually utilized by the cattle and buffaloes and whatever common nutrients are consumed by sheep and goat are expected to be balanced by the return of nutrients in the form of meat and milk for human consumption. The same is the case with the swine kept in the rural areas and hence, they have not been considered in determining the balance of total nutrients available and required for the Province.

Nutrients available for the livestock. Attempt has been made in Table IV to arrive at a figure for the nutrients available for livestock. Grain : straw ratios used for the calculation are 1 : 1 for gram and ' other food crops ' (mainly pulses), 1 : 1·5 for rice and *mandua*, 1 : 2 for wheat, barley, *kodon* and *sawan*, 1 : 2·5 for *bajra* and maize and 1 : 3 for *jowar*. Foreign [Morrison] figures have been used for computation wherever Indian [Sen, 1938] feeding values were not available. Kellner's [1926] value for millet straw was used for *jowar, bajra, mandua, sawan* and *kodon* straws. Value of straw from ' other food crops ' which consists mainly of pulses, has been assumed as equal to that from gram. *Jowar, bajra, guar* and maize are the main fodder crops during *kharif*, whereas the crops during *rabi* are lucerne, *senji*, oats and peas. The nutritive value of the cultivated fodders has been taken as the average of these crops. The yield of hay from the grazing land had to be assumed, but as suggested by the Royal Commission [1928] the yield from ' culturable waste ' and ' not available for cultivation ' has been taken as three-fourths and one-fourth of that from the forest area. The nutrients have been culculated on the basis of prime spear grass hay.

TABLE IV

Nutrients available for livestock

Crop	Area in thousand acres	Yield of feeds and feeding stuffs per acre (lb.)	Total yield of feeds and feeding stuffs in thousand tons	Digestible protein		Net energy		Dry matter per cent
				Per cent	Total in thousand tons	Therms per 100 lb.	Total therms in thousands	
Rice	6,902	1200 straw	3,998	nil	nil	28·8	2,385,000	90
Wheat	7,397	1800 ,,	5,944	,,	,,	24·0	3,196,000	90
Barley	4,130	1714 ,,	3,161	0·90	28·4	23·6	1,670,000	90
Jowar	2,590	1284 ,,	1,485	1·60	23·8	23·9	794,900	90
Bajra	3,040	1018 ,,	1,381	1·60	22·1	23·9	739,600	90
Mandua	99	450 ,,	20	1·60	0·3	23·9	10,600	90
Sawan	712	600 ,,	191	1·60	3·1	23·9	102,100	90
Kodon	1,307	600 ,,	350	1·60	5·6	23·9	187,400	90
Maize	2,424	2105 ,,	2,278	2·20	50·1	27·0	1,377,000	90
Gram	6,080	568 ,,	1,541	2·20	33·9	10·8	372,700	90
Other food crops	4,715	400 ,,	842	2·20	18·5	10·8	203,700	90
Potato	138							
Fruit and vegetable	471							
Sugarcane	1,865	5000 tops	4,163	0·80	33·3	16·3	1,520,000	20
Fodder crops	1,604	16400 green	11,740	1·83	213·9	10·7	2,806,000	25
Linseed	151	315 cake	21	24·40	5·2	79·9	37,950	90
Til	264	160 ,,	19	38·34	7·2	80·3	33,950	90
Mustard	185	408 ,,	34	27·61	9·3	75·3	56,900	90
Groundnut	116	229 ,,	12	45·60	5·4	81·1	21,630	(decorticated)
Cotton	311	340 seed	47	11·24	5·3	82·5	87,400	90
Rice bran		10 per cent of rice	247	7·13	17·6	50·6	279,000	90
Total	44,501		37,174		483·0		15,881,830	
Grazing land								
Forest	2,860	1000 hay	1,277	0·76	9·7	22·4	640,600	90
Culturable waste	9,569	750 ,,	3,205	0·76	24·4	22·4	1,608,000	90
Not available for cultivation	9,534	250 ,,	1,064	0·76	8·1	22·4	533,800	90
Total			5,546		42·1		2,782,400	
Total of crops, by-products and grazing			42,737		525·1		18,664,230	

The excess of 8,934,550 thousand therms fuel left from human food, as mentioned previously, is equivalent to the total of 630, 138, 350, 1156 and 90 thousand tons of barley, *bajra*, maize, gram and 'other food grains' respectively and the expected quantities of wheat bran and pulse *chunies*—the grains being approximately 40, 25, 39, 75 and 10 per cent of the total production. This extra food can supply 2,257 thousand tons dry matter, 252·3 thousand tons digestible protein and 4,481,960 thousand therms net energy for feeding the livestock as shown in Table V.

Table V

Nutrients available from extra feed

Particulars	Quantity in thousand tons	Dry matter in thousand tons	Digestible protein		Net energy	
			Per cent	Total in thousand tons	Therms per 100 lb.	Total therms in thousands
Barley	630	567	7·90	49·8	80·7	1,139,000
Bajra	138	124	7·82	10·8	90·3	279,100
Maize	350	315	7·40	25·9	90·0	700,600
Gram	1,156	1,040	11·41	131·9	78·1	2,022,000
Other food Crops	90	81	20·00*	18·0	78·2	157,700
Wheat bran	59 (20 per cent of wheat)	54	9·96	5·9	55·1	73,360
Pulse *chunics*	84 (12·5 per cent of other grains)	76	11·95	10·0	58·4	110,200
Total	2,507	2,257	..	252·3	..	4,481,960

* On the basis of Morrison's figure for peas

Increase necessary in the milk yield. For human consumption some protein of animal origin is essential and a vegetarian should get at least one-fifth of the protein from this source [*Health Bulletin*, 1937]. If this amount is to be met from milk alone, the requirement should be 13 oz. per head per day.

Considering the non-vegetarians of the province, if the level of average intake of this protective food is aimed at 8 oz.†, the yield will have to be increased by 1,544 thousand tons as shown in Table VI.

Table VI

Increase necessary in milk production

	Population	Total yield (tons)	Consumption per head per day (oz.)
Actual	..	3,047,000	5·3
Required	56,346,456	4,591,000	8·0
Increase necessary	..	1,544,000	2·7

The amount of this extra milk provided for human consumption will release some food for the use of the livestock, which is equivalent to the total of 24,592 and 75 thousand tons of *jowar* maize and 'other food grains' respectively and contains 371 thousand tons dry matter, 36·1 thousand tons digestible protein and 734,700 thousand therms net energy. On the other hand, the production of 1,544 thousand tons milk of cow and buffalo will require 80·3 thousand tons digestible protein and 1,283,000 thousand therms net energy. Taking all the points into consideration the position of the food supply for livestock can be summarized as in Table VII.

† Suggested in a private communication by Major G. Williamson, Animal Husbandry Commissioner with the Government of India.

TABLE VII

	Dry matter in thousand tons	Digestible protein in thousand tons	Net energy therms in thousands
Requirement	74,294	3,489·6	69,128,997
Requirement to increase the milk yield by 1,544,000 tons	..	80·3	1,283,000
Total requirement	74,294	3,569·9	70,411,997
Available from feeding stuffs	33,968	525·2	18,664,230
„ „ food crops	2,257	252·3	4,481,960
„ „ „ for extra milk supply	371	36·1	734,700
Total available	36,596	813·6	23,880,890
Shortage	37,698	2,756·3	46,531,107
Shortage—per cent requirement	51	77	66

Table VII shows that for balancing the requirement of the livestock, the quantity of available dry matter should be doubled. Digestible protein and net energy should also be increased by 77 and 66 per cent respectively.

How to make up the shortage. From the data it can be observed that about 4·8 times more crude protein and 2·9 times fuel are necessary for 30 million livestock than what is required for 56 million human population when the average digestibility of protein by the livestock is assumed as 60 per cent and the ratio of fuel to net energy is 2 : 1. But it is surprising to note that, in view of the relative requirements, the area under fodder crops is as low as 3·6 per cent of the total area under crop.

Even with poor yield of the crops, besides the present 1·6 million acres, fodders can be raised on another 10 million acres, the area which produces the grains in excess of human requirement. Fodder crops provide much more nutrients for the livestock than the grain crops. For comparison the yield of the nutrients per acre of some important grain and fodder crops have been tabulated in Table VIII.

TABLE VIII

Yield of nutrients per acre from different crops

	Green			Grain			Straw			Total per acre		
	Dry matter (lb.)	Digestible protein (lb.)	Net energy (therms)	Dry matter (lb.)	Digestible protein (lb.)	Net energy (therms)	Dry matter (lb.)	Digestible protein (lb.)	Net energy (therms)	Dry matter (lb.)	Digestible protein (lb.)	Net energy (therms)
Food crops—												
Rice	..	..	..	720	45·4	451	1,080	*Nil*	311	1,800	45·4	762
Wheat	..	..	..	810	68·9	685	1,620	*Nil*	389	2,430	68·9	1,074
Barley	..	..	..	771	60·9	622	1,542	13·9	264	2,313	74·8	886
Jowar	..	..	..	385	22·4	299	1,155	18·5	276	1,540	40·9	575
Bajra	..	..	..	366	28·6	331	916	14·7	219	1,282	43·3	550
Maize	..	..	..	758	56·1	682	1,895	41·7	512	2,653	97·8	1,194
Gram	..	..	..	511	58·3	399	511	11·2	55	1,022	69·5	454
Other food crops	..	..	..	360	72·0	282	360	7·9	39	720	79·9	321
Oats-grains after first cut	2,050	215·7	1,025	295	20·9	209	738	6·6	172	3,083	243·2	1,406
Groundnut	..	..	..	422	85·3	394	..	..	..	422	85·3	394
Fodder crops												
Jowar	5,125	102·5	2,307	..	..	..	..	..	..	5,125	102·5	2,307
Bajra	4,100	177·1	2,089	..	..	..	..	..	..	4,100	177·1	2,089
Maize	4,100	182·6	2,182	..	..	..	..	..	..	4,100	182·6	2,182
Lucerne	9,840	1737·0	4,428	..	..	..	..	..	..	9,840	1737·0	4,428
Berseem	4,305	579·8	2,440	..	..	..	..	..	..	4,305	579·8	2,440
Guar	3,075	163·6	812	..	..	..	..	..	..	3,075	163·6	812
Senji	4,100	410·0	1,558	..	..	..	..	..	..	4,100	410·0	1,558
Soyabean	2,563	327·9	1,343	..	..	..	..	..	..	2,563	327·9	1,343
Oats	4,100	431·3	2,049	..	..	..	..	..	..	4,100	431·3	2,049

From Table VIII it is observed that amongst the food crops maize gives the best return, net energy per acre being 1,194 therms. Wheat, barley and rice come next in order. But all the main fodder crops provide at least twice the amount of net energy than maize as a grain crop. The return of protein is also much higher from fodder crops. An acre of lucerne provides the maximum nutrients but it occupies the land throughout the year. The rotation berseem and *chari* or *bajra* comes next followed in order by the rotations oats and *guar*, soyabean or maize, and *senji* and *jowar*, maize or *bajra*. With the above considerations it is possible to produce from the extra 10 million acres which is approximately equivalent to net 6·39 million acres when double cropping is taken into account, the amount of nutrients as given in Table IX in excess if fodder crops are raised.

TABLE IX

Extra nutrients if fodder crops are raised

	Rabi					*Kharif*				
	Area in thousand acres	Dry matter in thousand tons	Digestible protein in thousand tons	Therms in thousands		Area in thousand acres	Dry matter in thousand tons	Digestible protein in thousand tons	Therms in thousands
Lucerne	1,500	6,589	1,163·0	6,642,000	Lucerne	..	..	..	..
Berseem	2,500	4,804	647·4	6,100,000	*Jowar*	1,250	2,861	57·2	2,883,000
					Bajra	1,250	2,289	98·9	2,616,000
Oat	2,000	3,661	385·1	4,098,000	*Guar*	400	549	29·2	324,680
					Soyabean	600	687	87·8	805,800
					Maize	1,000	1,831	81·3	2,182,000
Senji	390	714	71·4	607,700	Maize	390	714	31·7	851,300
TOTAL	6,390	15,768	2,266·9	17,447,700	TOTAL	4,890	8,931	386·1	9,662,780
Total during the year by raising fodder crops	*6,390*	*24,699*	*2,653·0*	*27,110,480*					
Total when food crops are grown	*6,390*	*2,628*	*288·4*	*5,216,660*					
Extra as a result of fodder crops	..	*22,071*	*2,364·6*	*21,893,820*					

It is observed that it is possible to make up the deficiency in the amount of protein from the area which can be spared from food crops with suitable fodder crops. But, 15,626 thousand tons of dry matter and 24,637,287 thousand therms remain still to be provided. Since grains supply more energy weight for weight, it usually becomes necessary to include grains in the ration to meet the energy requirement within the limited capacity of dry matter intake. Hence, either the area under cultivation should be increased by utilizing the culturable waste or the yield improved for providing more grains for balancing the energy.

In certain localities it may not always be possible to increase the area under cultivation and in others it may be undesirable, as it would either reduce the grazing land or increase soil erosion. On the otherhand, in view of the present poor yield, it appears to be more feasible to increase the yield. Additional production can also release more area for raising fodders if necessary.

In case it is possible to increase the yield by 50 per cent, 2,429·6 thousand tons of protein and 85,956,825 thousand therms fuel can be made available, with the present cropping arrangement. After providing for the human population the excess nutrients can provide 9,495 thousand tons dry matter, 1,061·0 thousand tons digestible protein and 18,850,000 thousand therms net energy for feeding livestock. The balance of nutrients is shown in Table X.

TABLE X

Balance of nutrients with 50 per cent increase in yield

	Dry matter in thousand tons	Digestible protein in thousand tons	Net energy in thousand therms
Balance from the food crop	9,495	1,061·0	18,850,000
From feeding stuffs (50 per cent increase)	50,902	787·7	27,996,345
From food crops due to extra milk supply	371	36·1	734,700
Total available for livestock	60,768	1,884·8	47,581,045
Total requirement for livestock	74,294	3,569·9	70,411,997
Shortage	13,526	1,685·1	22,830,952

Even with 50 per cent increase in the yield there is a deficit of 13·5 million tons of dry matter, 1·7 million tons of digestible protein and 23·0 million thousand therms of net energy. This indicates that some feed or a crop which can provide the required amount of digestible protein and energy within 13·5 million tons of dry matter is needed to balance the requirement. Grains like barley *bajra* and maize contain the nutrients approximately in the proportion required. But, even with the increased rate of yield more than 24 million acres of extra area will have to be cultivated to provide 13·5 million tons of dry grains. On the otherhand, the shortage can nearly be made up if 8 million acres of the present *rabi* area can be sown with berseem even when allowance is made for the nutrients from the corresponding *rabi* crops as shown in Table XI. Where facilities are available, this area can easily be spared for berseem. With 50 per cent increase in yield, the need of the human population can be met from 25·0 million acres out of the total 44·5 million acres leaving 19·5 million acres for raising feeds or fodders according to the requirements. The yield per acre of *rabi* food crop has been taken as 1,621 lb. dry matter 73·3 lb. of digestible protein and 709 therms net energy, the average of wheat, barley, gram and 'other food crops' for arriving at the net extra return from berseem crop.

TABLE XI

Nutrients from berseem and rabi *food crops*

	Dry matter in thousand tons	Digestible protein in thousand tons	Net energy in thousand therms
Yield from eight million acres of berseem (50 per cent increase)	21,948	2,958·8	27,888,000
Yield from eight million acres of rabi food crop (50 per cent increase)	8,680	392·9	8,512,000
Extra yield due to berseem	13,268	2,565·9	19,376,000
Shortage	13,526	1,685·1	22,830,952
Balance	..	+580·8	—3,454,952
Balance—percentage of total requirement	..	+16·3	—4·9

The deficiency of energy is only 4·9 per cent of the total requirement. The excess of digestible protein by 16·3 per cent may be necessary due to the loss of protein while conserving the green feeds.

The total production during the year has only been considered while balancing the nutrients. But without proper conservation of the green fodders as hay or silage, for even distribution of nutrients throughout the year, full utilization can not be obtained.

It is evident from the above that, for properly feeding the livestock at least one-fifth of the cultivated area should be put under suitable fodders even when the yield of crops is increased by 50 per cent of the present figure.

Another important factor also requires attention. The excessive amount of nutrients necessary can be reduced without impairing the production with better efficiency of animals.

Table III shows that the digestible protein and energy requirement of milch cows and buffaloes for maintenance alone are 4·8 and 6·6 times more than what is required for production of milk. The amount of digestible protein and net energy for maintenance are 755·0 thousand tons and 16,753,800 thousand therms respectively as compared to 158·2 thousand tons and 2,554,000 thousand therms for production. With higher rate of milk yield the number of animals can be reduced for the same amount of production, thereby reducing the maintenance requirement to a great extent. With better efficiency more bullock power can also be obtained with less number of bullocks. This point has already been discussed by Das Gupta [1945].

Summary

It is estimated that out of 1,619·7 thousand tons of protein and 57,304,550 thousand therms fuel available from food grains, seeds and milk, about 1,219·3 thousand tons of protein and 48,370,000 thousand therms fuel are required per year for 56 million human population of the province. The balance which is equivalent to 2,257 thousand tons dry matter, 252·3 thousand tons digestible protein and 4,481,960 thousand therms net energy, can be spared for the livestock.

The total requirement for maintenance, growth, production of milk or work for 30,208,247 livestock, excluding sheep, goats and swine, is 74,294 thousand tons of dry matter, 3,489·6 thousand tons digestible protein and 69,128,997 thousand therms net energy per year. The present total yield of 3,047 thousand tons of milk can provide, on an average, 5·3 oz. per head per day. If the consumption is to be increased to 8·0 oz. about 1,554 thousand tons more milk will have to be produced and the total requirement for livestock will increase to 3,569·9 thousand tons digestible protein and 70,411,997 therms net energy.

The amount of nutrients available for feeding livestock is 36,596 thousand tons dry matter, 813·6 thousand tons digestible protein and 23,880,890 thousand therms net energy which is only 49, 23, and 34 per cent respectively of the requirement.

Fodder crops provide nearly twice as much net energy as grain crops per acre. Though 4·8 times more protein and 2·9 times fuel is necessary for 30 million livestock than what is required for 56 million human population, only 3·6 per cent of the total cropped area is under fodder. With the present condition of poor yield, it is not possible to meet the requirement even if the total area corresponding to the excess of grains after providing for human population is put under the best fodder crops.

The shortage can be met if the yield can be increased by 50 per cent and about one-fifth of the total area is put under fodder crops, mainly legumes.

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CATTLE FOOD POSITION IN DELHI PROVINCE

By Ram Swarup, I. D. D., Assoc I. D. I., Officer-in-Charge, Animal Husbandry Bureau, Imperial Council of Agricultural Research

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IN this note an attempt has been made to estimate and compare the amount of animal feed produced in Delhi* with the amount needed to put the livestock in the optimum nutritional condition for full production.

The computation has been based on assumptions which appear to be, and probably are, reasonable but might nevertheless be erroneous and on data of present production and animal population which are not unquestionable. The note has been prepared more with a view to demonstrate the method which might be employed for making similar estimates rather than to propound an established fact.

It is found that the cattle feed production in Delhi province and the amounts actually required to insure proper feeding are as indicated in Table I.

TABLE I

Cattle feed position in Delhi province

	Amounts available in tons	Amounts required in tons	Amounts required to be imported or by which production should be increased	Percentage of requirements available
Bhoosa	32,030	71,580	39,550	44·7
Green fodder crops and maize (dry fodder basis)	106,024	84,398	..	125·6
Straw of gram and pulses	19,100	30,141	11,041	63·4
Oil cake	1,290	17,338	16,048	7·4
Gram	17,000	20,642	3,642	82·4
Bran and pollards	542	15,858	15,316	3·4

The statement above is based upon the calculations and assumptions as stated below:

ASSUMPTIONS

A. (*a*) All stock except horses and ponies get all their requirements during July and August from grazing.

(*b*) Milch stock get half their requirements for two months from grazing in addition to (*a*) above.

(*c*) Young stock and dry stock get all their requirements during July to November both inclusive and half their requirements for the rest of the year from grazing.

(*d*) All bulls and work animals get all their requirements from grazing during July and August. The rural bullocks get in addition half their requirements for half the remaining period but the urban bullocks get half their requirements for two months only from grazing.

(*e*) Idle stock get all their requirements from grazing.

(*f*) Horses and ponies get half their roughage requirements from grazing all the year round.

B. (*a*) It is assumed further that cows weigh 900 lb., bullocks 1200 lb. and horses 600 lb.

(*b*) Cows are Hariana and give three-fourth of the average milk output of farm Harianas. Buffaloes are Murrah and should give the average output of farm Murrahs. (This works out to 7 lbs. and 9 lb. milk per day—5 per cent and 7 per cent fat.)

(*c*) That all work animals do on the average about four hours work per day for the year.

*Presented before the Crops and Soils Wing Meeting held in December, 1945, at Delhi.

Calculations

The cattle of Delhi are enumerated under the following headings in the Livestock Census Report for 1940 :

(*a*) *Males over 3 years*	(*b*) *Females over 3 years*	(*c*) *Young stock*
(i) Breeding bulls	(i) Breeding cows in milk	(i) Birth to one year
(ii) Work animals	(ii) Breeding cows dry	(ii) One year to three years
(iii) Not used for breeding or work	(iii) Breeding cows not calved	
	(iv) Cows used for work	
	(v) Cows not used for work or breeding	

Of these categories (*a*) (iii) (*b*) (v) and (*c*) (i) may be ignored for our purpose, categories (*a*) (i), (*a*) (ii) and (*b*) (iv) may be grouped together. (*b*) (iii) may be grouped with (*c*) (ii). We have then, groups in cattle and also in buffaloes with their populations as indicated in Table II.

Table II

Groups in cattle and buffaloes with their population

Class	Cattle (Zebu)	Buffaloes
A Breeding bulls and work animals		
(1) Rural	30,675	579
(2) Urban	3,777	287
B Cows in milk	8,649	18,442
C Dry cows	12,860	6,486
D Young stock over one year	24,122	14,214
E Horses and ponies	7,715	..

The feed requirements for a balanced ration of the above categories are given in Table III. In practice the usual substitutions can be made, e.g. barley and oats *bhoosa* instead of wheat *bhoosa* etc. All the quantities are given on dry feed basis.

Table III

Feed requirements for a balance ration of different categories

	Bhoosa	*Jowar* or *bajra* fodder	Gram or legume fodder	Oil cake	Gram	Bran
Bulls and work animals	12	12	5	2·5	2·0	2·0
Cows in milk (Zebu)	8	8	4	1·5	2·0	1·0
Cows in milk (buffalo)	6	10	4	2·0	2·0	2·0
Cows dry	10	10	4	1·0	..	..
Young stock	4	4	..	2·0	2·0	..
Horses & ponies	2	8	..	..	4·0	4·0

Balanced according to *Imperial Council of Agricultural Research Miscellaneous Bulletin* No. 25 (Reprint 1945).

Based on the above balanced ration, and the assumptions listed before the annual requirements of cattle feeds will be as given in Table IV.

TABLE IV

Annual requirements of cattle feed in tons

	Population	No. of days to be stall fed	Wheat or other *bhoosa*	*Jowar* or *bajra* fodder	Gram or legume fodder	Oil cake	Gram	Bran
Bulls and work animals								
Rural	31,254	152	25,464	25,464	10,610	5,305	4,244	4,244
Urban	4,074	275	5,988	5,988	2,495	1,247	998	998
Cows in milk (Zebu)	8,649	275	8,496	8,496	4,248	1,593	2,124	1,062
Cows in milk (buffaloes)	18,442	275	13,584	22,640	9,056	4,528	4,528	4,528
Cows dry	19,346	108	9,330	9,330	3,732	933	..	..
Young Stock (1-3 years)	38,336	108	7,464	7,464	..	3,732	3,732	..
Horses	7,715	Fodder 182 365	1,254	5,016	..	..	5,016	5,016
Total			71,580	84,398	30,141	17,338	20,642	15,858

The following figures of production in Delhi province are based on the estimates given in the *Proceedings of the Second Meeting of the Central Fodder and Grazing Committee* of the Imperial Council of Agricultural Research pages 41-56 :

Fodder crops, maize and *kadbi*	106,024	tons
Rice wheat and barley straw	32,030	,,
Fodder from gram and pulses	19,100	,,
Oilcakes and cotton seed	1,290	,,
Gram (total production)	17,000	,,
Bran	542	,,

It thus appears that Delhi produces only 84·5 per cent of the fodder requirements and 35 per cent of the concentrate requirements of her livestock population. If the livestock is to be kept in a nutritional condition for optimum production, it is necessary to make the balance good.

SUITABILITY OF SUGARCANE TOPS FOR SILAGE MAKING

By T. M. Paul and M. C. Rangasamy, Imperial Dairy Research Institute, Bangalore

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SUGARCANE is very extensively cultivated in India, the estimated land under sugarcane for 1944-45 being 4,134,000 acres. The yield of sugarcane varies from province to province, but the average yield of millable cane is estimated to be about 20 tons per acre. The yield of sugarcane tops available in India is roughly 22 per cent of the millable cane which amounts to a little over four tons per acre. This amounts to 16,536,000 tons, and is likely to be on the increase. Thus very large amounts of sugarcane tops are produced in the sugarcane tracts during the sugarcane milling season. A comparatively negligible portion of it, is being utilized as fodder for cattle while the rest of it is allowed to wither and dry on the field and subsequently utilized as fuel for feeding the furnaces during the concentration of the sugarcane juice or simply burnt off on the fields. Thus it is clear, that a very large proportion of the sugarcane tops produced in India is allowed to go to waste. If some profitable means of utilizing this is found, that will certainly add to the income of the sugarcane cultivator. According to 'Morrison Sugarcane tops make satisfactory forage for livestock' and our own experience is that cattle relish them when they are fresh, but when they are wilted and a bit dry, cattle do not relish them much, possibly due to the coarse nature of sugarcane tops and leaves. Again due to the local and seasonal concentration in the production of sugarcane tops, one cannot expect that any considerable portion of it will be utilized as cattle feed in the fresh state. Therefore some suitable method of preserving the sugarcane tops with or without processing has to be evolved, if all the sugar tops is to be utilized as fodder. By making silage perhaps, its fodder qualities may improve and the cattle may relish it just like any other roughage. Moreover silage making is a method of preservation of fodder for seasons of fodder shortage. Thus, if silage could be successfully made out of sugarcane tops and if cattle could be fed on it safely, it may go a great way to relieve the fodder shortage in the country and thus contribute to the solution of the milk-problem to a certain extent. Both in America and Japan, sugarcane as well as sugarcane tops and bagasse have been successfully fed to cattle as silage and otherwise. But so far no data is available as to the suitability of the sugarcane tops for silage making under the Indian conditions and how far the indigenous breeds of cattle will relish it as fodder. Therefore an attempt was made at the Imperial Dairy Research Institute to study this problem.

Experimental

Sugarcane tops were procured locally in as fresh a condition as possible. Daily about a ton of the material was brought and cut into $\frac{1}{2}$ to $\frac{1}{4}$ inch bits in a chaffing machine and filled into five ft. cube silage pits dug in the ground. On the first day the sugarcane tops were filled into silo No. I without the addition of any thing else, second day's sample was filled into silo No. II with the addition of molasses at the rate of 40 lb. per ton diluted with an equal amount of water and the third day's sample was mixed with an equal amount of a mixture of guinea and Napier grasses and filled into silo No. III. During the filling of the silo pits, a representative sample was taken for analysis. Care was taken to tramp down the chopped matter very well, particularly at the sides of the pit to drive out as much air as possible. The pits were filled upto two feet above the ground level and covered with some *ragi* straw upto one foot thick. These were then covered with mud so as to give a hemispherical shape on the top. Finally the surface was plastered with a wet mixture of mud and cowdung so that the pit was almost air and water tight. All the pits were daily examined for any crevice on the surface and the crevices that appeared were immediately repaired. After three months the pits were opened, the top layer of *ragi* straw was removed and the silage examined for texture, flavour etc. A representative sample was removed from each silo pit for proximate analysis. Table I gives the results of the analysis before and after making the silage.

TABLE I

Results of analysis before and after silage making

Constituents	Silo I		Silo II		Silo III	
	Cane tops	Silage	Cane tops	Silage	Cane tops and grass mixture	Silage
Dry matter	36·8	33·3	33·3	27·0	29·5	41·5
Crude protein	5·7	4·6	5·3	3·5	5·9	4·5
Ash	5·2	5·4	4·7	5·3	8·4	10·5
Crude fibre	30·5	38·8	31·9	33·1	42·6	45·6
Ether extract	0·7	0·8	0·6	0·7	0·8	0·9
Acidity	1·4	3·2	1·3	2·3	1·5	4·5

Feeding trial. Twelve cows of almost the same stage of lactation, milk yield and body weight were selected from the Institute herd and were divided into four groups of three each. The normal concentrate ration of the animals consisted of a mixture of ricebran, gram, gram husk and groundnut cake in the ratio of 4 : 1·5 : 2·0 : 2·5. This was fed at the rate of one lb. for every two lb. of milk yielded by the animal. The roughage ration consisted of 55 lb. of green grass (mixture of guinea and Napier grasses) and three lb. of *ragi* straw per animal per day. The silage was fed in part replacement of the grass mixture in the normal roughage ration at the rate of two lb. silage for every three lb. of grass. Group I received silage from sugarcane tops alone, group II silage from sugarcane tops with molasses and group III received silage from sugarcane tops with mixture of farm grasses, while group IV remained as control, receiving the normal ration. On the first day of the feeding 15 lb. of the normal grass ration was replaced by 10 lb. of the particular silage in each experimental group. On the second day 22½ lb. of the grass ration was replaced by 15 lb. of the particular silage in each experimental group. Thereafter 20 lb. of the particular silage was fed in replacement of 30 lb. of the grass mixture of the roughage in each of the experimental groups for one month. The control animals received the normal ration throughout the period of experiment. Also the dry matter of the roughage was maintained at the same level in all the groups throughout the period. The milk yield and fat percentage of the milk was observed at every milking and the average body weight of the animals were noted every week. The animals did not show any reluctance to consume 20 lb. of either of the silages per day, but they seemed to like the silage made with the addition of molasses better than that made without molasses and the silage made with a mixture of farm grasses was liked best by the animals. The molasses silage had a better aroma, while mixed grass silage was more acidic and softer also in texture. Tables II—IV give the daily milk yield average fat content and the body weight of the animals respectively.

TABLE II

Daily milk yield of the animals in pounds during the experimental period

Days	Group I			Group II			Group III			Group IV		
	1	2	3	1	2	3	1	2	3	1	2	3
Initial	17·0	18·5	18·0	12·0	13·5	14·6	13·0	12·0	12·5	15·0	14·0	13·5
1	18·5	18·5	18·2	11·7	13·5	14·2	13·0	11·7	12·2	15·2	13·7	13·5
2	17·2	18·2	18·0	11·7	13·5	14·0	13·0	12·2	12·0	15·0	14·0	13·2
3	17·5	18·5	18·2	12·0	13·5	14·2	13·0	12·0	12·5	15·0	14·2	13·0
4	16·8	18·0	17·7	12·2	13·2	14·0	12·7	11·7	12·5	14·7	14·0	13·2

TABLE II—*contd.*

Daily milk yield of the animals in pounds during the experimental period

Days	Group I			Group II			Group III			Group IV		
	1	2	3	1	2	3	1	2	3	1	2	3
5	17·0	18·5	17·7	12·6	13·5	13·7	12·7	11·7	12·0	14·7	14·0	13·0
6	17·2	18·2	18·0	11·7	13·2	13·7	12·5	11·7	12·2	15·0	13·7	13·0
7	17·5	18·5	17·7	11·7	13·0	13·7	12·5	11·5	11·7	14·7	13·7	13·2
8	16·7	18·0	17·5	11·7	13·2	13·5	12·2	11·5	12·0	14·5	13·5	12·7
9	16·5	17·7	17·7	11·7	13·2	13·5	12·2	11·2	11·7	14·2	13·5	12·7
10	17·0	17·7	17·5	11·5	13·0	13·5	12·2	11·5	12·0	14·2	13·7	13·0
11	17·2	17·7	17·7	11·5	13·0	13·7	12·0	11·0	11·7	14·0	13·5	13·2
12	16·7	18·0	17·5	11·7	13·0	13·7	12·0	11·2	11·5	14·0	13·2	13·0
13	16·5	17·5	17·2	11·2	12·7	13·7	11·7	10·7	11·7	14·0	13·5	12·7
14	16·7	17·7	17·2	11·5	12·7	13·5	11·5	10·7	11·5	14·2	13·2	12·5
15	17·0	17·5	17·5	11·2	12·5	13·5	11·7	10·5	11·7	14·0	13·0	12·5
16	16·7	17·7	17·2	11·7	12·7	13·2	11·5	10·7	11·5	14·0	13·0	12·2
17	16·5	17·2	16·7	11·2	12·7	13·2	11·0	10·5	11·5	14·2	13·2	12·0
18	16·0	17·0	16·7	10·7	12·7	13·0	11·2	10·5	11·2	13·7	13·0	12·2
19	15·7	17·2	16·5	10·7	13·5	13·2	11·0	10·7	11·0	13·7	13·2	12·5
20	15·7	17·2	16·2	11·0	13·2	13·5	11·5	10·7	11·0	13·5	13·0	12·0
21	15·7	17·5	16·5	10·7	12·7	13·5	11·2	10·7	11·2	13·2	12·7	12·0
22	15·5	17·5	16·2	11·0	12·5	13·2	11·0	10·7	11·0	13·5	13·0	11·7
23	15·7	17·2	16·2	11·2	12·0	13·0	10·7	10·5	10·7	13·7	12·7	11·5
24	15·2	17·0	16·0	10·7	12·2	12·7	10·7	10·7	11·7	13·7	12·7	11·5
25	15·2	16·7	15·7	10·7	12·0	12·5	10·7	10·5	11·0	13·5	12·2	11·7
26	15·7	16·7	15·7	11·0	11·7	12·7	10·5	10·7	10·7	13·2	12·5	11·5
27	15·5	16·5	15·5	10·7	11·7	12·5	10·7	10·5	10·7	13·5	12·7	11·2
28	15·0	16·2	15·7	10·5	11·2	12·2	10·2	10·2	10·7	13·2	12·5	11·5
29	15·2	16·5	15·7	10·5	11·0	12·0	10·7	10·5	10·5	13·0	12·7	11·0
30	15·2	16·2	15·5	10·7	11·2	12·2	10·5	10·2	10·2	13·2	12·7	11·2
31	15·0	16·2	15·5	10·5	11·2	12·2	10·7	10·2	10·0	13·0	12·5	11·2

TABLE III

Average daily fat percentage

	Group I						Group II						Group III						Group IV					
	1		2		3		1		2		3		1		2		3		1		2		3	
	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
Initial	4·4	6·0	4·0	5·3	5·2	6·0	3·9	4·7	4·0	5·1	3·7	4·3	4·3	5·2	4·1	5·0	4·0	5·1	4·0	5·0	4·0	4·6	3·9	4·5
1st week	4·5	6·0	4·1	5·1	5·3	6·2	4·1	4·8	4·2	5·3	3·8	4·2	4·2	5·0	4·2	5·3	4·0	5·2	4·1	5·1	4·2	4·7	3·9	4·6
2nd ,,	4·3	6·2	4·3	5·5	5·1	6·4	4·2	4·7	4·4	5·2	3·9	4·4	4·4	5·3	4·3	5·2	4·3	5·1	4·2	5·5	4·3	4·9	4·1	4·4
3rd ,,	4·7	6·3	4·2	5·3	5·0	6·1	4·4	5·1	4·3	5·3	4·1	4·7	4·3	5·2	4·5	5·4	4·2	5·3	4·2	5·2	4·4	4·8	4·3	5·1
4th ,,	4·8	6·2	4·4	5·5	5·5	6·4	4·4	5·0	4·4	5·2	4·0	4·9	4·5	5·3	4·4	5·3	4·4	5·5	4·3	5·6	4·5	5·1	4·5	5·2

M = Morning sample E = Evening sample

TABLE IV

Weight of the animals in pounds

Period	Group I			Group II			Group III			Group IV		
	1	2	3	1	2	3	1	2	3	1	2	3
Initial	721	687	595	643	590	605	685	593	635	795	780	765
1st week	715	678	587	648	597	601	698	608	642	796	788	763
2nd ,, ...	728	695	602	640	602	608	679	602	629	793	799	766
3rd ,, ...	723	684	611	643	595	600	684	595	632	802	778	772
4th ,, ...	719	691	599	638	598	610	691	599	638	794	782	768

DISCUSSION

The feeding of the silage to the cows does not seem to have affected their milk yield. There was no sudden change either way in the milk yield in any of the three experimental groups. But there was a gradual decline in the average daily milk yield in all the groups. The control groups also showed such a decline and hence this common fall in milk production has to be attributed to advance in both lactation and pregnancy. Even if the fall in milk yield of the experimental groups was due to the silage feeding, it is not statistically significant when compared to that of the control group.

The fat percentage of the milk also did not show any unusual change that could be attributed to the feeding of the silage. As is commonly observed, the morning milk contained less fat than the evening milk.

The body weight of the animals also remained unaffected throughout the experimental period. The general health of the animals were quite satisfactory. There was no digestive or any other disturbance during the period when the silage was fed.

In general the cows liked the silages made with molasses and with the mixture of farm grasses better than that made with cane tops alone probably because they were less dry and softer. There was no adverse effect noticed on the cows that could be attributed to the feeding of silage.

SUMMARY

Silage was made from sugarcane tops alone, sugarcane tops with molasses and sugarcane tops with a mixture of guinea and Napier grasses.

The molasses and mixed grass methods produced more palatable silage than that made from sugarcane tops alone.

The silages produced no adverse effect on the milk yield, fat output, general health and body weight of milking cows when fed to them in quantities upto 20 lb. each daily.

REFERENCE

Morrison (1944). *Feed and Feeding*

THE PREPARTURIENT MILKING OF DAIRY ANIMALS

By ZAL R. KOTHAVALLA and A. J. LAZARUS, Imperial Dairy Reserarch Institute, Bangalore

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THE milking of a cow before calving under normal circumstances is generally resorted to only when the animal is suffering from some udder trouble, particularly inflammation of the udder, which commonly occurs in high yielders and first calvers. As a rule this practice is objected to on mere sentimental grounds, as a measure counter to nature and because it is supposed to make calving more difficult. Neither the merits nor the demerits of preparturient milking have so far been put to a systematic test on a scientific basis.

Of recent years increasing attention has been given to this subject of premilking of cows. It has been claimed by its advocates that the practice greatly enhances the milk yield of the animal and also reduces the chances of subsequent udder troubles, such as often arise in heavy milkers. Espe [1938] states that the massaging of the udder is quite a common practice to prevent the congestion which frequently occurs at the time of parturition. Webber and Turner [1929] have studied the influence of milking pregnant animals before calving on their physical condition and the well-being of the progeny. Though these authors state that premilking increases the yield of milk after parturition, they discourage the practice on account of its having adverse effects on the secretion of colostrum. The investigations carried out by Sayer [1934] with the pedigree Sahiwal herd at Pusa is the only work done on the subject in India so far. This herd was established in 1904 and maintained under normal dairy conditions. In course of time a considerable amount of udder trouble arose in the herd, which seriously affected its performance. With view to remedying this evil the practice of massaging the udder and milking the cows before calving was introduced in 1932; after calving the animals were milked four times a day at equal intervals instead of only twice. Further, in the case of heifers the bag was massaged from about two months before calving and the animals were thoroughly accustomed to milking; when the milk flow started, all the milk was drawn out. After calving, the same animals were milked from 7 to 15 times a day until they began to let down all the milk. As a result of these trials a good many advantages have been claimed, the principal ones being that the udder troubles altogether disappeared and the milk yield of the heifers and cows increased on an average by about 47 per cent as compared with the pretreatment period.

Whatever be the claims made for the adoption of premilking, one outstanding disadvantage of the practice persists and requires further investigation, namely that the calf dropped by the premilked cow does not receive the valuable colostrum, but Sayer (*loc. cit.*) in this connection claims that where the premilking of animals is adopted in a herd, the precalving colostrum would be given to calves born to premilked dams but in case that is not available the calf should be given linseed oil. By this method he asserts that mortality amongst calves was decreased from 4·3 to 1·4 per cent within a year of the adoption of this practice. Dover and Siva Subramanian [1939] from an intensive study of the chemical analysis of colostrum, have drawn attention to the fact that the colostrum obtained from premilked animals is deficient in globulin and ash to a considerable degree and they conclude from their other observations that the practice of premilking animals is a harmful one from the point of view of rearing the newly born calf.

In view of these conflicting views it was considered that fresh evidence upon which the merits of the practice of preparturient milking could be judged was necessary and the investigation which is discussed below was undertaken.

EXPERIMENTAL

The investigation in question was carried out for two to three lactations on Sindhi cows and Sindhi, Gir and cross-bred heifers maintained at the Imperial Dairy Research Institute, Bangalore.

Premilking of Cows. (i) Twelve Sindhi cows were taken for the experiment and an equal number of animals were kept as controls. The animals in these two groups were nearly of the same age, weight and stage of lactation at the start of the experiment. All the animals were healthy

and free from udder and other organic trouble. (ii) The animals in the experimental group were taken to the calving shed about a month before they were due to calve and in addition to the daily grooming and brushing, etc., the udders were massaged three to four times a day. (iii) When the udders began to fill out with milk, which occurred usually about 10 to 15 days before calving, the milk was drawn. The maximum quantity of milk thus obtained never exceeded 6·5 lb. the usual average being 4·7 lb. per day. (iv) Four days after calving, the animals were removed to the milking byre and milked twice a day during the whole lactation. (v) The control animals were treated in a similar manner except that their udders were not worked up, nor was any attempt made to draw off milk before calving. (vi) Particulars regarding milk yields of the lactations before and during the experiment and the birth-weight of calves in respect of both the control and experimental cows, etc. are given in Table I. One of the cows in the experimental group died after completing one lactation before further observations could be made. A few animals had to be disposed of during the third year of the experiment for various reasons. (vii) A record of the increase in the monthly weight of some of the calves during the first period of experiment was kept and the figures obtained are shown in Table III. The calves of the experimental animals were fed on the milk drawn from the respective dams. No separate colostrum was fed to them as it was the intention to observe incidentally the effect of the preparturient treatment on the offspring.

TABLE I

Premilking of Sindhi cows

Serial No.	No. of animals	No. of lactations the animal was in at the start of the experiment.	Lactation milk yield previous to the start of the experiment in lb.	Lactation milk yield during the experimental period in lb.			Average milk yield during the experimental period in lb.	Sex and birth weights of calves born during the experimental period in lb.		
				Control						
1	236	3	2,089	1,277 (died after completing one lactation)			1,277	F. 38	—	—
2	248	3	2,402	5,862	6,379	6,127	6,061	F. 54	M. 55	M. 47
3	249	3	3,700	6,589	6,167	4,676	5,811	F. 42	F. 50	M. 45
4	251	3	2,776	3,777	4,127	4,402	4,102	M. 49	F. 46	F. 50
5	253	3	6,443	5,119	5,857	6,493	5,823	F. 55	F. 55	M. 52
6	256	2	5,519	4,235	4,426	5,061	4,574	M. 48	F. 40	F. 49
7	258	2	1,117	3,664	4,294	—	3,979	M. 45	M. 37	F. 40
8	259	2	2,340	2,524	2,489	2,056	2,356	M. 50	F. 40	F. 40
9	263	2	5,695	2,436	4,231	3,322	3,208	M. 49	F. 40	F. 38
10	270	2	4,704	6,441	5,092	4,740	5,424	M. 40	M. 49	M. 45
11	277	2	2,784	4,836	1,688	—	3,262	F. 50	F. 45	
12	279	2	2,872	3,060	3,142	3,542	3,260	M. 40	M. 52	M. 49
Average	—	—	3,554	—	—	—	4,105	Male: 47.0	Female: 44·0	
				Experimental						
1	233	4	3,394	2,611	3,529	—	3,066	F. 43	F. 50	—
2	238	3	3,769	5,357	4,755	4,969	5,027	M. 54	F. 45	F. 54
3	247	3	3,443	3,776	3,337	1,967	3,027	M. 47	F. 45	F. 38
4	252	3	4,100	4,123	4,845	3,578	4,139	F. 34	F. 40	F. 37
5	255	2	3,847	3,924	4,097	819	2,947	M. 46	F. 42	F. 38
6	257	2	4,466	3,955	4,574	3,464	3,998	M. 50	F. 40	F. 46
7	265	2	3,848	4,805	1,615	4,473	3,631	F. 45	—	—
8	269	2	4,639	5,121	2,670	4,657	4,149	M. 50	M. 48	M. 55
9	271	2	2,027	3,597	3,315	3,139	3,350	M. 65	M. 60	M. 47
10	275	2	759	5,482	3,228	3,889	4,150	F. 60	F. 50	M. 41
11	278	2	4,052	5,629	3,141	4,897	4,556	M. 50	M. 59	M. 55
12	282	2	4,301	4,424	4,257	—	4,340	M. 52	M. 55	—
Average	—	—	3,554	—	—	—	3,862	Male: 52·1	Female: 44·2	

Premilking of heifers. (i) Forty heifers belonging to the Sindhi, Gir and cross-bred herds were taken for study and after dividing them into control and experimental groups were subjected to similar treatment, as indicated above for the cows. In this case as the milking potentialities of the animals were not known it was rather difficult to evenly distribute high and low yielders. (ii) After the premilking was started, as in the case of cows, the daily maximum yield obtained was 4·5 lb., the usual average being about 2·5 lb. (iii) After calving the heifers were milked four times a day for a week and this was reduced to three times a day for the following four weeks. By this time the animals settled down and the milking was subsequently done only twice a day for the rest of the lactation. (iv) The obervations made in respect of the heifers are given in Table II. (v) It was found that out of 55 animals subjected to the preparturient milking treatment, four showed some udder trouble and about 20 animals were slightly nervous at the time of calving.

TABLE II

Premilking of Heifers

Serial No.	No. of the animals	Lactation milk yields during the experimental period (in lb.)			Average annual milk yield during the experimental period	Sex and birth weights of calves born during the experimental period		
		Sindhi heifers—Control group						
1 . .	310	1,721	—	2,936	2,328	M. 53	M. 50	F. 47
2 . .	313	2,503	—	3,375	2,939	M. 38	M. 45	—
3 . .	316	1,294	1,616	3,003	1,914	M. 48	F. 48	F. 48
4 . .	317	415	—	788	551	M. 40	F. 47	F. 33
5 . .	318	3,248	—	2,521	2,784	M. 40	F. 47	F. 30
6 . .	329	1,545	—	2,261	1,903	M. 42	F. 42	—
7 . .	331	205	—	322	263	M. 42	M. 47	—
8 . .	333	828	—	2,432	1,630	M. 45	M. 42	F. 47
9 . .	337	4,706	—	3,345	4,025	F. 40	M. 42	—
10 . .	339	2,542	—	2,170	2,356	M. 47	M. 40	—
11 . .	341	2,347	—	—	2,347	M. 42	F. 42	—
12 . .	343	4,475	—	—	4,475	F. 45	M. 50	—
13 . .	347	4,186	—	—	4,186	M. 38	—	—
14 . .	350	3,663	—	—	3,663	M. 42	F. 39	—
Average .	—	—	—	—	2,526	M. 44	F. 42	—
		Sindhi heifers—Experimental group						
1 . .	294	3,198	3,120	3,608	3,308	M. 30	F. 38	M. 52
2 . .	295	944, 1,560, 1,400	—	886	1,197	M. 49	M. 30	F. 37
3 . .	296	2,095	2,464	598	1,717	F. 47	M. 55	M. 45
4 . .	297	7,116	5,885	3,344	5,448	F. 38	V. 45	M. 60
5 . .	298	2,225	2,108	2,677	2,510	F. 44	M. 47	M. 50
6 . .	299	847	—	—	847	F. 40	—	—
7 . .	300	1,168	1,631	3,509	2,069	M. 53	M. 49	M. 57
8 . .	301	1,406	—	—	1,406	M. 39	M. 50	—
9 . .	302	2,739	3,734	3,669	3,381	F. 45	M. 53	F. 50
10 . .	303	225	1,674	1,747	1,215	M. 50	M. 43	F. 43

TABLE II—*contd.*
Premilking of Heifers

Serial No.	No. of the animals	Lactation milk yields during the experimental period (in lb.)			Average annual milk yield during the experimental period	Sex and birth weights of calves born during the experimental period		
		Sindhi heifers—Experimental group						
11	304	200	2,984	3,616	2,216	M. 55	M. 56	M. 47
12	307	659	1,777	—	1,218	M. 52	F. 44	—
13	309	258	154	—	206	M. 45	F. 41	—
14	312	1,156	—	—	1,156	M. 43	M. 41	—
15	314	940	943	3,127	1,670	M. 49	M. 57	M. 47
16	315	1,695	—	—	1,695	M. 48	F. 38	—
17	327	3,872	3,053	—	3,462	F. 44	F. 42	F. 56
18	330	2,956	3,002	—	2,979	M. 44	F. 57	M. 66
19	332	2,871	—	—	2,871	F. 40	M. 43	—
20	335	4,363	3,240	—	3,801	F. 42	M. 48	—
21	338	2,800	—	—	2,800	F. 40	—	—
22	340	3,945	3,971	—	3,958	F. 39	M. 43	—
23	344	1,889	1,968	—	1,968	F. 38	M. 39	—
24	346	2,702	—	—	2,702	F. 43	F. 54	—
25	348	3,395	—	—	3,395	M. 43	—	—
26	349	2,185	—	—	2,185	F. 45	F. 38	—
Average	—	—	—	—	2,360	M. 48	F. 44	—

TABLE IIB

Serial No.	No. of the animals	Lactation milk yields during the experimental period (in lb.)			Average annual milk yield during the experimental period	Sex and birth weights of calves born during the experimental period		
		Gir heifers—Control group						
1	44	3,604	3,851	—	3,727	F. 37	F. 47	F. 44
2	45	3,618	3,555	—	3,586	M. 55	F. 46	—
3	47	1,623	—	—	1,623	M. 56	F. 52	—
4	50	333	3,548, 3,224	—	2,368	F. 40	F. 52	F. 48
5	51	2,890	2,895	—	2,892	M. 50	F. 47	M. 51
6	54	3,646	2,446	—	3,066	M. 65	F. 50	F. 60
7	55	3,279	—	—	3,279	F. 50	—	—
8	57	3,255	2,764	—	3,009	M. 53	F. 58	—
Average	—	—	—	—	2,943	F. 48	M. 55	—
		Gir heifers—Experimental group						
1	34	4,761	3,134	2,911	3,602	M. 50	M. 64 F. 45	M. 54
2	36	2,486	2,675	—	2,580	F. 45	F. 48 M. 55	—
3	38	1,674	1,876	1,875	1,842	F. 50	F. 52 F. 54	M. 46
4	48	2,600	1,286	—	1,943	M. 60	M. 59	—
5	49	1,986	2,189	—	2,087	F. 37	F. 45 F. 47	—
6	53	1,876	1,143	—	1,509	M. 52	M. 54 F. 51	—
7	56	1,042	1,075	—	1,058	M. 56	M. 53	—
8	58	3,894	—	—	3,894	M. 50	M. 55	—
Average	—	—	—	—	2,304	M. 54	F. 47	—

TABLE IIB—*contd.*

Serial No.	No. of the animals	Lactation milk yields during the experimental period (in lb.)			Average annual milk yield during the experimental period	Sex and birth weights of calves born during the experimental period		
					Cross-bred heifers—Control group			
1 . .	600	2,829	—	—	2,829	F. 50	F. 53	—
2 . .	605	3,700	—	—	3,700	F. 47	F. 37	—
3 . .	607	6,647	8,964	—	7,805	F. 44	M. 50 M. 52	—
4 . .	611	3,857	3,547	—	3,702	F. 47	F. 50 M. 51	—
5 . .	616	6,259	7,084	—	6,671	F. 47	M. 70 M. 64	—
6 . .	618	3,968	4,829	—	4,828	M. 50	M. 63	—
7 . .	620	7,545	—	—	7,545	M. 56	M. 66	—
8 . .	622	6,999	—	—	6,999	M. 47	F. 55	—
9 . .	624	5,734	—	—	5,734	F. 58	F. 51	—
10 . .	626	7,291	—	—	7,291	M. 50	M. 60	—
Average .	—	—	—	—	5,710	M. 56	F. 49	—
					Cross-bred heifers—Experimental group			
1 . .	599	4,402	—	2,445	3,423	F. 60	M. 40	—
2 . .	601	3,015	—	—	3,015	F. 47	F. 54	—
3 . .	602	6,907	7,075	6,477	7,059	M. 62	F. 65 F. 52	M. 60
4 . .	603	3,640	3,665	3,644	3,650	F. 55	M. 61 M. 55	M. 56
5 . .	606	5,144	6,263	—	5,703	F. 54	M. 63	—
6 . .	609	3,594	4,215	—	3,904	M. 42	F. 58 F. 58	—
7 . .	610	3,242	2,480	—	2,861	F. 45	M. 57 F. 60	—
8 . .	621	6,166	—	—	6,616	F. 70	—	—
9 . .	623	5,948	—	—	5,948	M. 53	—	—
10 . .	625	4,593	—	—	4,593	F. 55	M. 70	—
Average .	—	—	—	—	4,677	F. 56	M. 55	—

All the experimental and control animals received the same treatments mentioned for each group. The animals were from the same herd, housed in the same place and looked after by one of the authors throughout. The calvings were distributed evenly throughout the year.

DISCUSSION

Premilking of cows. From Table I, in which are given the results of the experiment on the Sindhi cows, the following conclusions can be drawn :—(i) For the control group the average annual milk yield in the year previous to the start of the experiment was 3,554 lb. The corresponding average for the experimental animals was also 3,554 lb. The animals were either in their second or third lactation when this investigation started. (ii) The experiment was carried out for three years. The average yield for all the control animals during this period was 4,105 lb. This showed an increase of about 15·5 per cent over the yield in the pre-experimental period. This can be explained by the fact that the animals selected were either in their 2nd or 3rd lactation and therefore the increase, even in the control group, during the period of experiment was to be expected. (iii) The rise in the milk yield in the corresponding period for the animals in the experimental group was from 3,554 lb. to 3,862 lb. or an increase of only 8·7 per cent. This would indicate that the animals in this group did not fare so well as in the control group, and the preparturient treatment instead of helping to increase

the milk yield had just the reverse effect. (iv) From the last column in Table I, it will be observed that the average weight of all the male calves in the control group was 47 lb. and of the females 45 lb. The corresponding weights for the calves from the experimental group were for male calves 52 lb. and for female calves 44 lb. There was thus not much difference between the birth weights of the experimental and control calves. (v) A more significant difference is to be found when the weights of individual calves in the two groups are compared over a period of twelve months. This weight was recorded for some of the calves born during the first year of the experiment and the results obtained are shown in Table III. For the sake of comparison the figures for male and female animals are given separately. It will be seen that, for the female calves the average weight at birth in the control group was 50 lb. and at the end of the first year it increased to 325 lb. In the experimental group these figures were 41 lb. and 285 lb. respectively. In both the groups the male calves started at almost the same level but while the animals of the control group had an average weight of 341 lb. at the end of 12 months, the experimental animals weighed only 308 lb. This is a very significant difference.

TABLE III

Birth and monthly weights of some Sindhi calves during the first experimental year

	Serial No.	No. of animals	Weight												
			At birth	1st month	2nd month	3rd month	4th month	5th month	6th month	7th month	8th month	9th month	10th month	11th month	12th month
Male															
Control	1	1,541	48	67	90	119	145	174	203	230	262	294	315	336	359
	2	1,561	44	66	89	114	139	167	201	228	249	275	294	315	345
	3	1,472	38	52	73	97	120	142	169	196	220	242	267	280	306
	4	1,586	49	65	91	116	145	178	205	231	254	290	315	351	353
	5	8	49	73	94	117	143	170	201	232	258	275	—	—	—
	Average	—	45·6	64·6	87·4	112·6	138·4	166·2	195·8	223·4	248·6	275·2	297·7	315·5	340·7
Experimental	1	1,535	46	67	88	109	132	155	179	198	218	236	259	278	301
	2	1,536	54	70	92	112	141	168	184	201	228	251	272	302	324
	3	1,583	47	68	87	109	132	158	180	205	231	257	276	303	331
	4	1,585	50	66	83	103	124	146	171	193	217	239	260	287	312
	5	52	50	68	87	106	124	143	168	192	213	230	252	270	292
	6	42	42	56	71	90	106	122	139	158	179	201	226	256	286
	Average	—	48·6	65·8	84·6	104·8	126·5	148·6	170·1	191·1	214·3	235·6	257·5	232·6	307·6
Female															
Control	1	1,534	54	77	99	128	152	181	204	209	253	279	298	320	338
	2	1,569	42	61	88	118	148	172	197	223	249	271	294	316	335
	3	23	55	76	101	127	153	179	201	225	247	269	296	318	339
	4	131	50	62	74	93	115	138	163	185	209	230	251	269	288
	Average	—	50·25	69	90·5	116·5	142	167·5	191·25	210·5	239·5	262·25	284·75	305·75	325
Experimental	1	1,564	43	54	70	88	106	125	149	163	184	210	232	285	272
	2	1,565	45	56	79	105	129	147	173	191	212	234	251	276	298
	3	60	42	63	84	106	127	149	173	194	215	232	264	285	304
	4	4	34	45	58	74	91	119	145	162	181	202	222	243	266
	Average	—	41	54·5	72·75	93·25	113·25	135	160	117·5	198	219·5	242·25	265·5	285

Premilking of heifers. (i) In the case of heifers the general conclusions are very similar to those discussed above, for all the breeds studied. (ii) The choice of the animals for the control and experimental groups was made so as to ensure that the animals of the same age and weight were evenly distributed in the two groups. In spite of this, the average yields for the control animals were invariably higher than those for the experimental ones. (iii) In the experiment with Sindhi heifers, the annual milk yield for the control (over the whole period studied) was 2,526 lb., as against 2,360 lb. for the experimental animals. (iv) When the figures for only the first experimental year are compared, it will be found that the yields for the control and experimental groups are 2,399 lb. and 2,225 lb. respectively, indicating a slight set back instead of an improvement for the experimental animals. (v) Most of the Gir heifers were studied for three years consecutively. Here also the average annual yield of 2,304 lb. for the experimental animals was found significantly lower when compared to the figure of 2,943 lb. for the control group. (vi) The average annual milk yields in the case of cross-bred heifers for the control and the experimental groups over the whole period of investigation were 5,710 and 4,677 lb. respectively, thus corroborating the results obtained with Sindhi and Gir heifers.

The results of the experiment bring out very significantly the following points (i) Preparturient treatment in an intensive form and premilking of cows and heifers do not in any way tend to improve the milk yield of the animals so treated. On the other hand it has an adverse effect especially in the case of heifers. The animals studied here might be regarded as moderate yielders and the results may perhaps be slightly different in the case of heavy yielders. (ii) In the present studies the animals selected were in good health, having no udder trouble, but it is quite possible that the premilking treatment may prove of benefit where the animals are predisposed to such trouble as reported by Sayer [1934]. (iii) A very significant point brought out as a result of the present studies was the weak health of the calves born to cows which had been premilked. This is illustrated by Table III. (iv) In the case of heifers it was observed that during the whole period of study the death rate of calves in the control group was 5 per cent lower than that of those in the experimental group. (v) About 73 per cent of the calves that died in the experimental group did so during their first month of life ; death was mostly due to digestive trouble. This demonstrates the importance of feeding colostrum to new born calves. (vi) Another important feature noticed in the case of all the experimental animals (cows and heifers) was retention of the placenta. Probably premilking causes weakness of muscular contraction of the uterus at parturition with the result that the placenta is not expelled. (vii) It was found that premilking and frequent massaging of the udder involved a considerable increase in the cost of maintaining the herd. (viii) Sometimes animals used to frequent milking, when removed from the calving shed and put with the herd and milked only twice a day, tended to hold their milk. This can be attributed to the extra handling which the animals were accustomed to during the preparturient treatment.

Summary

The practice of milking Sindhi, Gir and cross-bred cows and heifers prior to calving was in no way advantageous. This treatment did not increase the milk yield to an extent which justified the extra cost involved. The calves of cows subjected to the treatment invariably failed to thrive as well as the controls and the mortality amongst them was very high.

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PRESERVATIVE EFFECT OF COMMON SALT ON THE DEVELOPMENT OF ACIDITY IN STORED GHEE

By Lal Chand Dharmani and Kartar Singh Lohara, Punjab Agricultural College, Lyallpur

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In a hot country like India where butter cannot be stored for a long time, it is melted into *ghee* to prevent decomposition. The dehydrated and clarified butter fat is stored either in tins or in earthen pots. Thousands of maunds of butter are melted into *ghee* every day in India and its storage is an important problem which requires careful attention.

Ghee, when stored for a long time, developes an acrid taste and unpleasant aroma which render it unfit for edible purposes and the acidity that finds its way into *ghee* during its preparation, is considered to be the most important, if not the main cause of these developments. Moisture in the presence of heat, light and air accelerates the development of acidity.

Banerjee and Doctor [1938] found that the acidity of *ghee* increased during storage from 4 to 36 days at 0°C. and room temperature. Paul Arup [1929] found that *ghee* stored in bottles fitted with metal screw caps and cork discs for six years showed a marked decrease in R.M. value and there was a close correlation between the free fatty acids formed and the loss in volatile acids.

Khubchandani [1939] studied the effect of citric acid and sodium citrate on flavour, aroma and keeping quality of butter and *ghee*. He added these compounds in the cream. The keeping quality was improved by citric acid.

Godbole and Sad Gopal found, in one sample only, that salt acts as a preservative against the action of light, air and moisture on the fat.

People in the Punjab adopt different methods of storing *ghee* in villages, the most common being to store it in tins or earthen pots. Common salt is used as a preservative either in the form of a big lump or as a powder.

With a view to studying the preservative effect of common salt on the development of acidity in *ghee* during storage the experiment described below was carried out.

Experimental

Ghee was prepared according to the country method which is described in Allen's *Commercial Organic Analysis* (Fifth Edition, Volume II, page 431). It was further clarified and made moisture-free in the laboratory by heating it over a sand bath.

Method of storage. Tins of about 1¼ lb. capacity with air tight covers were thoroughly washed with distilled water and dried in the air oven at 105°C. for 24 hours. *Ghee* was melted at 40°C. and 400 gms. of *ghee* were stored in each tin. Fresh distilled water was added to bring the moisture contents of the stored samples up to 0·5 per cent, 1·0 per cent and 1·5 per cent. Butter milk was used at the rate of 0·25 per cent only. Ordinary common salt was finely powdered and added at the rate of 2 per cent of the weight of *ghee*. The tins were covered with air tight covers and shaken vigorously for a sufficiently long time to insure dispersion of water and salt. Five samples of each treatment were kept. The experiment was arranged as follows :

Tin No.	Treatment
1 to 5	0·5 per cent moisture
6 to 10	1·0 per cent moisture
11 to 15	1·5 per cent moisture
16 to 20	0·5 per cent moisture and 2·0 per cent salt
21 to 25	1·0 per cent moisture and 2·0 per cent salt
26 to 30	1·5 moisture and 2·0 per cent salt
31 to 35	No treatment
36 to 40	0·25 per cent butter milk
41 to 45	0·25 per cent butter milk and 2 per cent salt

All these treatments were repeated using small earthen pots of almost of the same capacity 1¼ lb. and with earthen lids except that butter milk was used at the rate of 1·0 per cent and 2·0 per cent. Before filling the earthen pots the dispersion of water and salt was effected by putting melted *ghee* in a stoppered glass bottle and shaking it for some time. When the water and salt were well dispersed the sample was quickly poured in the pot and covered with a lid and cloth was wrapped over the mouth of the pot. The butter milk used was obtained from the College dairy and contained 0·2 per cent lactic acid acidity. All the stored samples were kept in the laboratory for about fifteen months. The temperature of the laboratory room varied from 34°F. minimum in winter to 114°F. maximum in summer. The earthen pots were placed in 500 c.c. capacity porcelain dishes.

The samples were tested periodically for acid value, smell, colour and taste. Acid value was determined by the method described in *A.O.A.C.* and the taste and smell were judged by five Research Assistants working in these laboratories.

Sampling for analysis. *Ghee* was melted by placing the tins in hot water (40°C. to 45°C.). The tin was shaken well and opened. The smell was noted. It was then filtered at 40°C. through filter paper (Whatman No. 1). The filtered sample was tested for smell, taste and acid value. The results are given in Tables No. I and II. They are the averages of duplicate readings and in certain cases triplicate determinations. The difference between the duplicate samples was very little and in many cases there was no difference at all.

TABLE I

Ghee Stored in 1¼ lb. tins on 28th July 1943 ; acid value 0·50

Date of analysis	Treatment	Acid value	Treatment	Acid value	Remarks
20-11-43	0·5 per cent moisture	0·62	0·5 per cent moisture and 2 per cent salt	0·62	No change in this smell colour, and taste
26-2-44		0·67		0·56	
10-4-44		0·73		0·73	Do.
10-10-44		0·73		·73	
20-11-44	1 per cent moisture	0·56	1 per cent moisture and 2 per cent salt	0·56	
26-2-44		·67		0·56	
10-4-44		·78		0·56	
10-10-44		0·73		0·73	
		Acid value original sample 0·35			
20-11-43	0·5 per cent moisture	0·56	1·5 per cent moisture and 2 per cent salt	0·56	Do.
26-2-44		·50		0·56	
10-4-44		0·45		0·62	
10-10-44		0·50		0·62	
20-11-43	Control	0·45			Do.
26-2-44		·45			
10-4-44		·56			
10-10-44		0·56			
25-11-43	0·25 per cent butter milk	0·39	0·25 per cent butter milk and 2 per cent salt	0·39	
10-4-44		0.73		0·73	Rancid smell, upleasant taste
10-10-44				1·18	

TABLE II

Ghee stored in earthen pots on 14th December 1943 ; acid value 0·67

Date of analysis	Treatment	Acid value	Treatment	Acid value	Remarks
15-5-44 . .	Control . . .	0·73			
13-10-44 . .		1·01			
15-5-44 . .	0·5 per cent moisture	0·90	0·5 per cent moisture and 2 per cent salt	0·67	
13-10-44 . .		1·40		·78	
15-5-44 . .	1 per cent moisture .	0·95	1 per cent moisture and 2 per cent salt	0·67	All the samples gave rancid smell, unpleasant taste after a period of about 5 months
13-10-44 . .		1·12		1·06	
15-5-44 . .	1·5 per cent moisture	0·78	1·5 per cent moisture and 2 per cent salt	0·56	
13-10-44 . .		·91		0·84	
15-5-44 . .	1 per cent butter milk	0·78	1 per cent butter milk and 2 per cent salt	0·95	
13-10-44 . .		·96		1·20	
15-5-44 . .	2 per cent butter milk	1·50	2 per cent butter milk	0·95	
10-10-44 . .		1·12		1·04	

Acid value was expressed as milligrams of KOH required to neutralize acids in one gram of *ghee*.

DISCUSSION OF RESULTS

Ghee was stored at room temperature in air tight tins on 28-7-1943. The acid value of the original sample was 0·50 (Table I). The increase in the acid value was very slow both in the salted and unsalted samples. After a period of 15 months the increase in acid value was from 0·50 to 0·73. There was no change in the aroma and taste of the samples. These results clearly indicate that *ghee* can be stored in air tight containers for a period of 15 months without undergoing any deterioration in quality. In the absence of air and light, moisture alone does not affect the keeping quality of *ghee*. The addition of salt, therefore, appears to be unnecessary.

Commercial samples of *ghee* always contain a certain amount of butter milk (*lassi*) and therefore, samples were also stored with 0·25 per cent butter milk, and it can be seen from the figures in Table I that this amount of butter milk did increase the acid value though to a slight extent. Salt in the presence of butter milk enhanced the development of acidity. These samples of *ghee* developed a rancid smell after a period of nine months.

Earthen pots. In earthen pots, the addition of salt checked the development of acidity, in *ghee*, but, as the pots could not be made air tight, air and heat played their part fully. Samples of *ghee* kept in earthen pots developed a very bad smell after a period of five months, inspite of the fact that the acid value was not very high. Besides the *ghee* oozed out of the pots during summer and considerable loss occurred. The actual losses were not determined but from the amount of *ghee* found in the dishes and on the outer surface of the pots, it was judged that the storage of *ghee* in earthen pots during summer months is not to be recommended.

SUMMARY

To study the preservative effect of common salt, *ghee* with varying amounts of moisture and butter milk (0·5 to 1·5 per cent) was kept in 1¼ lb. tins and earthen pots of the same capacity. Salt was used at the rate of 2 per cent of the weight of *ghee*.

The development of rancidity of *ghee* was judged by the characteristic aroma, acrid taste and increase in free fatty acids. Samples were tested periodically for 15 months. The results so for obtained show that a good quality *ghee* can safely be stored in air tight tins upto 15 months without showing any signs of deterioration. The use of common salt appears to be unnecessary.

It is not advisable to store *ghee* in earthen pots because the acidity developes rapidly and because the *ghee* oozes out of the pots during summer months and thus causes considerable loss.

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AN ACCOUNT OF TWO SPECIES OF LUNGWORMS FROM INDIAN GOATS *

By M. M. SARWAR, Department of Food, Government of India, New Delhi

(Received for publication on 15 December 1944)

(With three text-figures)

THE generic differentiation of species in the family Metastrongylidae is to a large extent based on the shape and arrangement of accessory pieces. These organs are elaborate and present varied features and this has resulted in a considerable confusion in adoption of proper terminology for these structures. Looss [1905] applies the term gubernaculum to a structure defined as ' thick brown chitinuous piece.....found embedded in the dorsal wall '. He thinks its function to lie in changing within certain limits the direction of the spicula when they are protruded. Hall [1921] uses the word gubernaculum for the more or less longitudinal structure in the dorsal wall of the cloaca towards the anterior end and the term telamon for the ' structure of variable form near the cloacal aperture '. Cameron [1927] applies the term telamon to the longitudinal paired accessory pieces and gubernaculum for the anteriorly placed unpaired ones. Gebauer [1932] uses the term in just the reverse order. Schulz, Orlow and Kutass [1933] call the whole arrangement as gubernaculum and designate the three parts as capitulum, corpus and crura. They apply the term telamon to a structure of variable form near the cloacal aperture. Such a structure has been pointed out in *Varestrongylus pneumonicus* by Bhalerao [1932], in *Pneumostrongylus calcaratus* by Monnig [1933] as a refractive piece of chitin which moves with the telamon gubernaculum of Gebauer [1932]. In *Pneumostrogmylus alpenae* Dikmans [1935] refers to the above structure as an unpaired accessory piece consisting of two proximally united deeply pigmented portions situated in the terminal portion of the body.

The arrangement of the accessory pieces suggests that capitulum, corpus and crura are intimately connected in function and modified accordingly in different species. In the species of the genus *Protostronglus* there is a capitulum and two pairs of corpus and crura arising separately. In *Protostrongylus austriacus* [Gebauer, 1932] the crura are fused at their anterior ends, in *V. pneumonicus* the corpus and crura are fused at their anterior and posterior ends respectively and capitulum has disappeared. In *Dictyocaulus filaria* (Fig I, a) the crura are fused and the corpus appear to degenerate while capitulum has disappeared. In the family Trichostrngylidae the appearance of gubernaculum suggests it to be the result of a more intimate adhesion of the crura. The capitulum and corpus, which are probably supporting structures to the crura when paired, disappear when the crura have fused as in the genera *Varestrongylus*, *Neostrongylus* and *Pneumostrongylus*.

In the following lines have been described two species, one under the genus *Varestrongylus* and the other under *Protostrongylus*. The *protostrangylus* species was collected from a goat once only while the *Varestrongylus* species was twice collected mixed with *V. pneumonicus*, the latter species predominating.

Varestrongylus capricola SARWAR 1944

The specimens which consist of three males and a number of females were collected from small bronchi of a goat at Mukteswar.

Male has a total length from 15 to 18 mm. and a width of about 0·14 mm. at about the anterior ends of the spicules. Oesophagus is 0·275 mm. long and 0·05 mm. broad and is lanceolate in shape.

The bursa (Fig I, b) consists of two lateral and a dorsal lobe and the rays are disposed as follows:

*The paper was read before the 31st session of the Indian Science Congress held at Delhi in 1944.

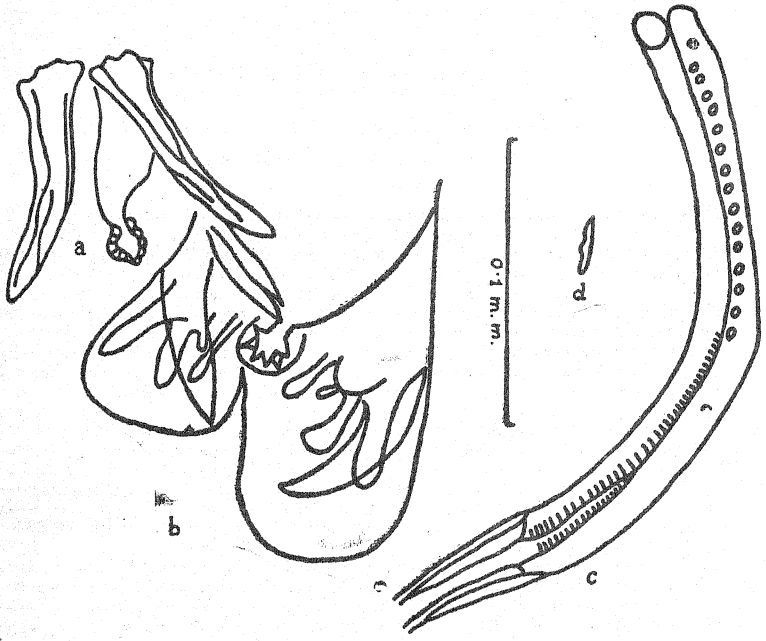

Fig 1. *a*. Spicules and Gubernaculum (*D. filaria*)..
b. bursa (*V. caprícola*).,
c. Spicules (*V. caprícola*) D. accessory Piece (*V. caprícola*).

Ventral rays are the largest and undivided. Antero-laterals is divergent from the fused medio- and postero-laterals. Externo-dorsal is a slender ray and arises independently. The dorsal ray is small and ends in apparently six papillae.

Spicules are 0·25-0·26 mm. long and are bent at about their middle. They are bifid at their posterior ends as in *V. pneumonicus* and are deeply pigmented. The cuticularized lamellae are coarser and heavier than those of the members of the genus *Protostrongylus*. It may, however, be remarked that measurements of spicules in 75 specimens of *V. pneumonicus* have shown them to vary from 0·42 to 0·45 mm. only.

The telamon consists of two yellowish chitinous pieces having indentations on their outer edges. Each piece measures about 0·025 mm. long and is situated near the cloacal aperture.

Females resemble those of *V. pneumonicus* except that the vulvar flaps are vestigial only. Length of oesophagus is 0·4 mm. and breadth about 0·065 mm. Vagina is 0·85 mm. long with the vulva situated in close proximity with the anus. Tail is about 0·05 mm. long. The eggs measure 0·065-0·07 × 0·03-0·04 mm.

Host : Hill goat (*Capra sibirica*)
Location : Bronchi
Locality : Mukteswar-Kumaun.

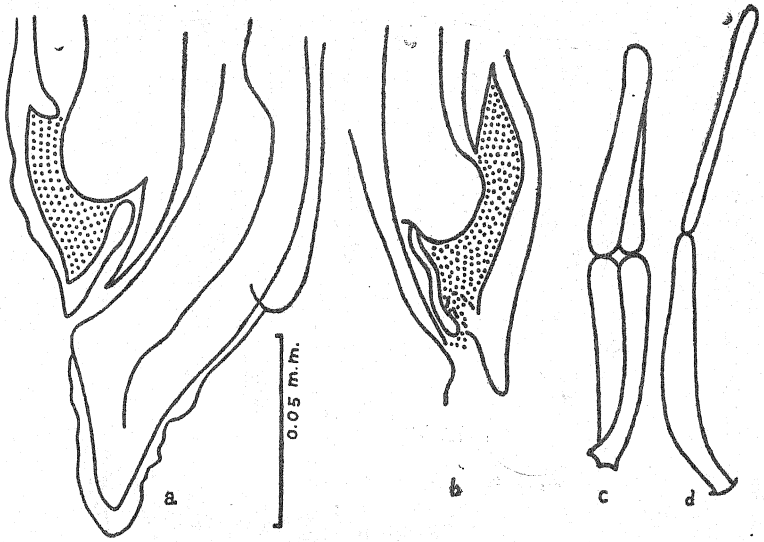

Fig 2. *a&b.* Posterior end of females (*V. capricola*), *c&d.* Gubernaculum (*V. pneumonicus*)

Several genera have been created under the family Metastrongylidae to accommodate numerous species with elaborate accessory structures of varied shapes and arrangement. An examination of material of various species can bring down a reduction in the known number of genera. *Pneumostrongylus* Monnig 1935 and *Neostrongylus* Gebauer 1932 are closely allied to *Varestrongylus* in their having fused crura and capitulum being absent. The spicules in the genera *Varestrongylus* and *Pneumostrongylus* have coarse cuticularised lamellae and telamon structures near the cloaca have been described. The species described in the preceding pages differs from the only other species of this genus by the absence of the spindle shaped gubernaculum described by Bhalerao [1932].

Protostrongylus indicus SARWAR 1944

Male : Total length is unknown as no entire specimen was collected. Breadth in the region of proximal end of spicule is about 0·17 mm. Rays of the bursa are disposed in pattern characteristic of the genus. Externo-lateral ray is separate and is widely separated from ventral. Medio-lateral and postero-lateral rays originate from a common stem and are separated in their distal parts. Externo-dorsal is relatively short. The dorsal is not studied in detail being folded beneath the body.

Spicules are from 0·42 to 0·5 mm. long and are characteristic of the genus with minute chitinoid fibrillations fringing their medical sides. The posterior end of spicule is devoid of fibrillations, is thinner and curved and presents a protrusion on the ventral side (Fig 3, d). The spicular sheath extends beyond the ends of the spicules for about 0·02 mm.

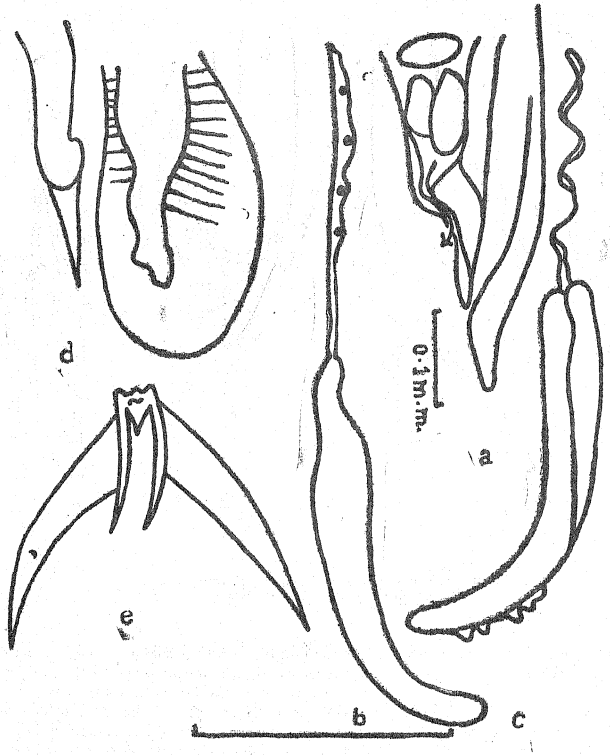

Fig 3. *Protostrongylus indicus a.* Posterior end, female; *b&c.* Paired accessory, *d.* Posterior of spicule, unpaired accessory piece.

The gubernaculum in general resembles that of the other species of the genus. The capitulum consists of a central head with two arms directed laterally and then posteriorly and two curved pieces with the keels directed dorso-laterally. Corpus consists of two slender lightly cuticularized parts each measuring about 0·12 mm. long. The crura are more deeply pigmented and more heavily cuticularized and each part measures about 0·145 mm. long.

Female. Total length unknown. Largest piece collected measures 20 mm. long and 0·2 mm. broad near the vaginal constriction. Vagina is 1·525 mm. long and is proceeded by a provagina. Tail is 0·09 mm. long and vulva is situated about 0·18 mm. from tail. Eggs are 0·08-0·1 mm. long and 0·035-0·038 mm. broad.

Host : Hill goat (*Capra sibirica*)

Location : Bronchi.

Locality : Lahore, Kangra (Punjab)

This species may be distinguished from *P. ocreatus*, *P. refescens*, *P. stilesi* and *P. raillieti* by the presence of a boot shaped accessory piece in the first and the presence of teeth on the posterior parts of crura in the latter three species. In *P. macrotis* and *P. capreolus* capitulum is absent. *P. austriacus* has proximal ends of crura fused. *P. rushi* lacks a provagina. In *P. rupicaprae* the curved arms of capitulum are directed laterally and the characteristic curved pieces with keels are absent. The posterior end of spicule is devoid of fibrillations, is thinner and curved and presents a protrusion on the ventral side (Fig, 3, d). A characteristic of *P. indicus* is the presence of a long vagina.

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Explanations to Illustrations. Plate I., a. Spicules and gubernaculum (*D. filaria*)., b. bursa (*V. capricola*) ; c. Spicules (*V. capricola*), d. Accessory piece (*V. capricola*)

Plate II. a. & b. Posterior end of female (*V. capricola*)., c. & d. Gubernaculum (*V. pneumonicus*).

Plate III. *P. indicus*., a. posteri or end., b. & c. distal portion of paired accessory piece., d. posterior end of spicule., e. unpaired accessory piece.

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ORIGINAL ARTICLES

CONJUNCTIVITIS OF SHEEP AND GOATS IN INDIA

By J. F. Shirlaw and N. S. Sankarnarayan, Imperial Veterinary Research Institute, Izatnagar

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(With Plate I)

SPECIFIC conjunctivitis of sheep and goats is known to exist in North and South Africa, the French Congo, the Levant, France, parts of the United States of America, in Uruguay and in Australia and New Zealand. Coles [1931] defined the cause of the condition in S. Africa as a new species of *Rickettsia* for which he proposed the name *R. conjunctivæ*, and this ætiological finding is accepted in those countries where the disease exists. According to Donatien and Lestoquard [1939] the disease is highly infectious and presumably air-borne, spreading rapidly through flocks and herds through the medium of ocular and nasal discharges. One eye is usually affected, exceptionally both, the chief signs of the disease being intense conjunctivitis with lachrymation and photophobia. The conjunctivæ of the swollen lids show either sparse or numerous fine granulations, and within a few days of the onset of the disease, muco-pus collects at the inner canthus of the affected eye. In spite of the sudden and somewhat alarming early signs of the disease, it is essentially benign in nature and the great majority of cases tend to recover spontaneously within ten days or so from the onset of the affection. In some cases, however, keratitis develops and pannus-like lesions are seen, *i.e.* invasion of the cornea by vascular stems infiltrating from the corneo-sclerotic margin. Corneal opacity is a fairly common sequel in these cases, and, if it be accompanied by ulceration, there is always the danger of a secondary bacterial infection of the inner eye.

Diagnosis is said to be relatively straightforward. The disease is usually easily transmitted to healthy sheep or goats by rubbing a little of the infective eye discharge over the healthy eyeball, preferably after mild scarification of the conjunctiva. The incubation period is 2—3 days. The causal organism, *R. conjunctivæ*, is pleomorphic, and spherical, semi-elliptical, and cocco-bacillary forms, the latter possessed of a central clear zone, are seen within the conjunctival cells. These forms stain a light blue or lilac in Giemsa preparations and are Gram-negative. The parasite measures 0·4—1·5μ and the average size of cocco-bacillary forms is about 0·6μ. In some cases, horseshoe forms of the parasite predominate within the cells, and these are considerably larger, measuring up to 2·0μ. The infection of the epithelial cells may be massive, the majority being densely packed with organisms, or clusters of the organism may be situated at the angles or margins of cells; or, the cell infection may be sparse, with only a few organisms scattered throughout it. A few small clumps or stray organisms may occasionally be found outside the cells, possibly liberated through rupture of the cells, and a few may be found within polymorphonuclear leucocytes which are generally present, but phagocytosis is, on the whole, feeble. There are two main fallacies to be guarded against in diagnosis. Rickettsia-like organisms, so-called *Rickettsioides*, are not infrequently seen in the conjunctival cells of healthy sheep and goats. These organisms are considerably larger than *R. conjunctivæ*, and are not so pleomorphic but spherical, if of somewhat irregular contour, and are usually scanty within the conjunctival cells. The organism is apparently feebly pathogenic. The second fallacy is that pigment granules are commonly seen within the conjunctival cells of sheep and goats, whether healthy or affected with the disease. However, these granules are easily differentiated from rickettsiæ by their golden-brown colour.

Observations and results

In January, 1941, the Veterinary Investigation Officer, Bihar, drew attention to a similar disease of sheep and goats in that province. This Officer obtained some preliminary evidence as to its transmissibility but on repeated tests, found 'that quite frequently the instillation of mixed ocular and nasal discharge did not reproduce the disease, even after a lapse of twelve days'. Early work at this Institute indicated that the disease was a specific rickettsial conjunctivitis and this finding was recorded in the *Annual Report of the Institute* [1941, as yet unpublished]. Since then, two papers have appeared—by Nanda and Abdussalam [1943] and Abdussalam [1944] —which indicate the

existence of rickettsial conjunctivitis in sheep and goats in the Punjab. The present paper outlines the work undertaken by us on behalf of the Veterinary Investigation Officer, Bihar.

Two goats were used in the test of material received from the January 1941 outbreak of disease in goats. The left eyeball of each goat was anaesthetised with 1 per cent cocaine solution, the corneal surface lightly scarified, and a few drops of suspected infective ocular discharge rubbed in. The disease could not be transmitted and no rickettsiæ were demonstrable in conjunctival films of field cases, stained Giemsa and Wolbach-Pinkerton. The disease again appeared in sheep in Bihar in October, 1941, and two sheep were received at Izatnagar as described below.

Sheep 1. This animal was affected with a severe, bilateral conjunctivitis with keratitis and pannus formation, the cornea of both eyes bulging and being quite opaque. No granulations were seen within the conjunctivæ of the lids, nor could rickettsiæ be demonstrated within the conjunctival cells. However, numerous polymorphs and Gram-positive diplococci, many phagocytosed, were evident in such concentration as to suggest a pure bacterial infection. The animal was destroyed and both eyes dissected. The anterior chamber contained a few fibrin shreds and the aqueous humour was slightly cloudy, but neither cells nor organisms could be detected on film examination. The lens, ciliary body, iris and inner structure of the eye appeared normal, and the vitreous was clear. Sections of the eyeball taken from the *ora serrata* of the retinal coat through the ciliary body, iris and cornea, after formalin fixation and staining by haematoxylin and eosin, showed irido-cyclitis and interstitial keratitis with intense eosinophilia, strongly suggestive of a parasitic infection. However, no nematode parasites could be detected in sections stained by Giemsa or Lœffler. The vitamin A liver reserve of this case was within normal limits. With material from this animal, two sheep, with apparently normal eyes, were inoculated in the anterior chamber of the eye with a few drops of mixed aqueous-vitreous humour. No reaction occurred in either animal over 85 days of observation.

Sheep 2. Both eyes were equally affected, the eyeballs bulging, and the cornea cloudy, save at the margin where pannus-like lesions were noted. No rickettsiæ were observed in Giemsa-stained films of the conjunctival epithelium, while by Gram's stain polymorphs were seen to be very numerous with diplococcal organisms similar morphologically to those seen in the first case. Cultures on blood agar from the conjunctiva, after swabbing with warm normal saline, yielded a Gram-positive diplococcus morphologically similar to the organism seen on direct film examination, and a smooth strain of *Bac. subtilis*. Two sheep were inoculated immediately under the bulbar conjunctiva of the left eye with two drops of a 48-hour serum broth culture of the coccus isolated, which was also liberally rubbed over the conjunctival surfaces of the same eye. No reaction occurred over a period of two months. With two other sheep, the bulbar conjunctiva of the left eye was lightly scarified under cocaine anaesthesia and the ocular discharge from Sheep 2 well rubbed in. Observation over 82 days failed to reveal any lesions. Also, two healthy sheep were kept in close contact in the same shed with Sheep 2, but neither contracted the disease during 82 days contact; incidentally, flies were numerous in the shed during this time. During the interval entailed by these transmission tests in sheep, very considerable improvement occurred in the eyes of Sheep 2, and after about ten weeks the only visible lesion was some haziness of the cornea and increased vascularity at the corneo-sclerotic margin: the intra-ocular tension had also abated. This phase was dramatically interrupted by a sharp and severe attack of conjunctivitis affecting both eyes, but no granulations were seen within the bulbar or palpebral conjunctivæ. Films from both these surfaces, however, stained by Giemsa and thionin blue, showed on this occasion unmistakable rickettsiæ within the majority of the conjunctival cells, either densely packed or disposed in tight clusters within the cytoplasm (Plate I, fig. 1).

The morphology and staining affinities of these organisms corresponded precisely with the original description of *R. conjunctivæ* Coles, 1931. At the same time, the signs of bacterial infection, originally noted in Sheep 2, again came into prominence in addition to the rickettsial infection. We were disappointed in our further efforts serially to transmit the disease to the eyes of healthy sheep.

EXAMINATION FOR RICKETTSIAE OF NORMAL EYES OF SHEEP, GOATS AND HILL CATTLE

A flock of 36 sheep and a herd of 29 goats maintained at the Institute were examined for evidence of ocular disease; with one exception, a goat affected with a unilateral, purulent conjunctivitis, none showed any sign of active or latent conjunctivitis, keratitis or granulations within the conjunctival membrane suggestive of rickettsial infection. Conjunctival films from 32 of the goats (including the case of purulent conjunctivitis) and 10 of the sheep were examined for rickettsiae. These were demonstrable within the conjunctival cells of every case. The extent of the infection was variable; in a few cases, it was well marked, up to 60 per cent of conjunctival cells harbouring the organism. In most cases, the organism was found in about 10 per cent of the cells. In four goats, the infection was light and some search was necessary to demonstrate the organism. The extent of the infection within the cells was variable in individual films. Some cells were densely packed with organisms (Plate I, fig. H), or they were present in clusters chiefly at the margins of cells. Occasionally, a few small clumps were found extracellularly, apparently liberated by the degeneration of infected cells. However, cell degeneration was not a conspicuous feature, and even a massive cell infection seemed well tolerated. The morphology and staining affinity of the organism conformed in detail to the classic description of *R. conjunctivae*. Neutrophils were occasionally seen in conjunctival films, and in a few cases Gram positive diplococci, many within the phagocytes. In the solitary case of conjunctivitis, rickettsiae were evident within the conjunctival cells but this finding was overshadowed by an apparent bacterial infection. Whilst in some films, neutrophils were present and apparently unaccompanied by bacteria, we did not consider that such cells were a concomitant feature of the infection; and, when present, they appeared to have no numerical relationship with the extent or intensity of the infection. In two of five hill cattle examined, 30 per cent of the conjunctival cells were infected with rickettsiae; in the remainder, cell infection was light but definite. No morphological or tinctorial differentiation could be established between the cattle and the goat organism.

EXAMINATION OF CASES OF HUMAN TRACHOMA

This was undertaken to compare the morphology and staining affinities of the goat organism with the human one. Eight cases were selected for examination, one an early active infection, three advanced and chronic, and four old and active. Rickettsiae were determined within the conjunctival cells in every case, the intensity of the infection varying in different cases, apparently independently of the clinical activity of the condition. No distinction was possible between the human and the goat forms of the parasite.

DISCUSSION

The scope of this investigation has been limited. Only on two occasions have we been notified of cases of conjunctivitis in sheep and goats in India. Enquiries from Veterinary Investigation Officers in the provinces (other than the Punjab) indicate that the disease has not been observed and a careful survey is obviously called for. During the war years, a large number of goats and sheep have passed through veterinary inspection at meat dehydration plants and army depots concerned with the collection and dispatch of these animals for the supply of meat in the eastern theatre of war. We had recently an opportunity of visiting one of these goats and sheep collecting depots where among thousands of animals we saw no evidence of the disease. The traffic in goats and sheep during the war years throughout northern India to these collecting depots has been so enormous that the animals in the depots have been a fair random sample of the goat populations in this part of India. We have had reports from the depots of every disease to which goats and sheep are subject; yet, only on one occasion have we had a report of conjunctivitis. A diagnosis of rickettsial conjunctivitis was made on examination of conjunctival films from a few cases, but this small outbreak cleared up before a field investigation could be undertaken. We have also seen a few scattered cases of keratitis among several thousand goats passing through a large meat dehydration plant in the North West Frontier Province, but nothing in the nature of an outbreak of the disease; indeed,

the incidence of the affection was so insignificant as to occasion no comment by the local Veterinary Officers and it would certainly have passed unnoticed had we not been particularly on the look out for it. Nor have we encountered the affection in the many hundreds of goats used in experimental work at the Institute over the last four years.

In our limited investigation into the aetiology of conjunctivitis and keratitis of sheep and goats in Bihar, we arrived at the conclusion that the condition had much in common with the similar disease described in these animals by Coles [1931], and that the cause was *R. conjunctivæ*. It is true that the disease we investigated was clinically similar to that described by Coles, and that *R. conjunctivæ* was identified in one of the sheep from Bihar and in several cases of the same disease in goats occurring at an army depot in 1945, but we are now by no means convinced of the aetiological relationship of these organisms. There are two main reasons for our doubt. Organisms which cannot be differentiated from *R. conjunctivæ* on morphological characters and staining affinities have been demonstrated within the conjunctival cells of every goat and sheep and of the few bovines that we have examined, and, with one exception, the eyes of all the animals selected for examination were perfectly healthy. We have no doubt that, if we extended our examination, the finding would be practically universal. In the second place we failed to infect the conjunctival membrane of normal sheep with ocular discharge from a frank case of conjunctivitis in a sheep from Bihar in which *R. conjunctivæ* was identified within the conjunctival cells. This experience confirmed the work of the field workers in Bihar and is contrary to the experience of workers in other countries on rickettsial conjunctivitis in small ruminants. One possible explanation of our experience is that the infection is really ubiquitous and latent in the sheep and goats that we have worked with, even if there are no apparent sub-clinical lesions.

Abdussalam [1944] found rickettsiæ similar to *R. conjunctivæ* within the conjunctival cells of a buffalo affected with conjunctivitis, but makes no comment on the examination of healthy eyes of the same animal species. He also records that, on examination of the eyes 24 of Malvi goats, chronic trachoma-like lesions were found in every case and rickettsiæ identified within the conjunctival cells, although there was no outward appearance of eye affection such as keratitis. He considers that these animals had suffered from a more acute form of the disease in their kidhood, and that in the adult this had reached a stage of chronicity. No systematic examination, however, of kids in the area was undertaken. It is also noteworthy that no transmission experiments with materials from the Malvi goats could be carried out, as '*unaffected goats could not be made available*'. It is not quite clear from this statement whether all the goats in the area were affected with specific conjunctivitis, or simply whether *R. conjunctivæ* infection could be determined within the conjunctival cells of these goats. If the latter, it agrees with our own observations. In the eyes of the normal goats and sheep which we examined, and in which we found rickettsiæ conforming in type to *R. conjunctivæ*, we have seen no signs of persistent and chronic trachoma-like lesions. According to information received, sub-clinical trachoma is a prevalent affection among the Indian population, and, even in apparently normal eyes, can be detected by slit-lamp examination of the vessels of the limbus. It may well be that a more careful examination of the eyes of goats and sheep on these lines would give a more accurate interpretation of the finding of *R. conjunctivæ*, within the conjunctival epithelium of these animals though, on naked eye examination, there is no evident abnormality. It may well be that the latency of the animal disease is similar to what has been observed in the human population. In this connection, it seems worth emphasising the statement of Beveridge [1942] that, although it has not been possible to prove the aetiological role of *R. conjunctivæ* in contagious conjunctivitis of goats and sheep, there is strong justification for accepting it as a working hypothesis. If, however, this position be accepted in respect of frank clinical cases of the disease in which the organism can be identified, what interpretation can be vouchsafed for the identification of the parasite in cases where no clinical disease exists ?

There is one last point. Contagious conjunctivitis of sheep and goats has been described as a superficial infection, and yet, although little work seems to have done on the essential pathology of the condition, there is some evidence that the deeper structures of the eye may be involved. Thus, Coles [1931] describes lesions of irido-cyclitis. It is not clear whether this lesion is considered a direct

sequel of an inward extension of a superficial rickettsial infection, but the genesis of the lesion is one that requires complete elucidation. As stated, we encountered this lesion in one case examined pathologically.

SUMMARY

1. A sporadic disease of goats and sheep clinically comparable with rickettsial conjunctivitis [Coles, 1931] exists in Bihar and has been detected, in a minor outbreak, in an army live-stock depot in India. An organism identified as *R. conjunctivæ* occurs within the cells of the conjunctival epithelium of such cases.

2. An organism indifferentiable in morphological characters and staining affinities from *R. conjunctivæ* was detected within the conjunctival cells of 32 goats, 10 sheep and five hill cattle selected at random, and in which, with one exception, *viz.* a goat affected with purulent conjunctivitis, the eyes showed no outward sign of disease.

3. An identical form of rickettsia was seen within the conjunctival cells of eight cases of human trachoma examined.

4. Lesions of irido-cyclitis were determined in one clinical case in a sheep.

5. The disease could not be transmitted to sheep with outwardly healthy eyes by means of ocular discharge from a diseased animal or by close contact in a small shed over a period of 82 days.

6. It is doubtful whether the finding of *R. conjunctivæ* in cases of conjunctivitis in the Indian goat and sheep has real ætiological significance.

ACKNOWLEDGEMENT

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FIG. 1. *R conjunctivae* within epithelial cells of goat affected with conjunctivitis (Leitz $\frac{1}{12}$ objective, 8 ocular)

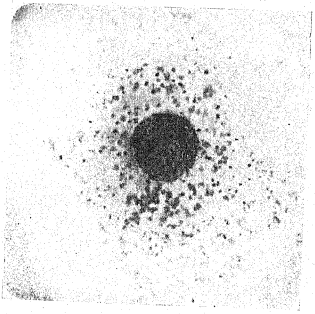
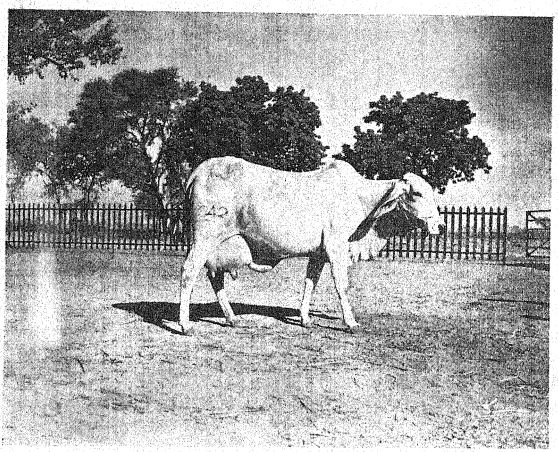


FIG. 2. *R. conjunctivae* within epithelial cells of unaffected goat (Leitz $\frac{1}{12}$ objective, 10 ocular)



A TYPICAL THARPARKAR COW

Mapji– By bull Maharaj out of cow Phijji. Mapji is a second calver, gave 7,009 lb. milk containing 5 per cent fat in her first lactation of 306 days. Phijji gave 10,232 lb. in her third lactation and has given 48,428 lb. milk in six lactations and is now in her seventh.

EFFECT OF FOUR TIMES MILKING AND HANDLING ON THE YIELD OF MILK IN COWS OF THE THARPARKAR BREED

By C. H. Parr and S. Sen, Imperial Agricultural Research Institute, New Delhi

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(With Plate II and three text figures)

THE frequency of milking has considerable effect on the secretion of milk in dairy cows. Ragsdale *et al.* [1924] observed that the longer the interval between milkings, the less the speed of milk secretion per unit of time. They found that if the amount of milk secreted during the first hour were represented as 100 per cent, the amount secreted in each succeeding hour would be approximately 95 per cent of that secreted during the previous hour. Woodward [1931] held that relief from the pressure of milk within the udder as a result of frequent milking allowed secretion to proceed more freely. Hammond [1936] found that the retardation of the rate of secretion and the final cessation of milk production were caused by the pressure created by the accumulated milk rather than the chemical effect of its constituents within the udder. Ispe [1941] has stressed the necessity for intermittent periods of intramammary pressure in order to stimulate milk secretion and advocates that the maximum milk production can be secured by frequent milking and thus preventing an accumulation of milk in the udder. Ispe and Hammond are also of the opinion that the possibility of milk or fat being secreted into the blood stream during periods of high intramammary pressure appears improbable. Bartlett [1929] showed that the yields of milk and fat at morning milkings after a long night interval, although greater in amount, were not in proportion to the interval of time between the respective milkings. He attributed the cause to reabsorption of milk taking place during the long night interval. To avoid this depression and to obtain optimum results he suggested the shortening of the long night milking interval.

Experiment with Tharparkar cows

An experiment to compare four and two times milking on Tharparkar cows maintained at the Karnal Sub-station of the Imperial Agricultural Research Institute was started in 1939. Twenty-four cows were selected for this purpose. Most of the cows had completed their first lactations when the experiment was started. As one cow died, data were available pertaining to eleven cows under four times milking and twelve cows under two times milking. The cows under four times milking were milked and 'handled', *i.e.*, the udder was massaged before calving for about ten minutes every day for a period of 15 to 20 days and regularly milked once a day as soon as milk secretion started, following the practice introduced by Sayer [1934] in the Sahiwal herd at Pusa and subsequent continued with the same herd at Delhi as a standard practice.

Rationing. Roughages were fed *ad lib.* in the form of *bhusa* (bruised oat and wheat straw), green sorghum, green cowpeas, green berseem, sorghum silage and berseem hay to provide the nutrients required for maintenance of animals of weights varying from 750 to 1,200 lb. Concentrates were fed in the proportion of one pound concentrates for every two pounds of milk yield. The composition of the concentrate ration was generally as follows :—crushed oats 48 per cent, crushed gram 32 per cent, crushed groundnut cake 12 per cent and crushed linseed cake 8 per cent. To this mixture was added sterilised bone meal and common salt each at the rate of 1½ lb. per 100 lb. of the mixture. The above gives a concentrate ration which provides 68·00 per cent starch equivalent and 13·71 per cent digestible protein, *i.e.*, 0·34 lb. of starch equivalent and 0·068 lb. of digestible protein per pound of milk against the standard requirement of 0·316 lb. of starch equivalent and 0·051 lb. of digestible protein per pound of milk containing five per cent fat [Sen, 1938].

Statistical Analysis of the Data

In their pre-experimental first lactations, eight cows were under two times milking, while another set of eleven cows were under four times milking. Of the remaining four cows, three had completed

three lactations and one cow four lactations when they were taken into the experiment—two under four times milking and two under two times milking.

In the statistical examination of the data, the average milk yields per day (wet period only) of the first set of eight cows as shown in Table I are first considered.

Table I

Average milk yield (wet period) in lb. per day of eight cows milked twice a day in their pre-experimental first lactation

Name of cow		During pre-experimental period		During experimental period				Mean	
				2nd Lactation		3rd Lactation			
Milked 2 times under experiment	Milked 4 times under experiment	1st Lactation Milked 2 times		Milked 2 times	Milked 4 times	Milked 2 times	Milked 4 times	2 times milking	4 times milking
1	2	3	4	5	6	7	8		
Urji . .	Lani . .	19·98	16·47	Aborted	18·17	19·89	18·65		
Larzi . .	Utakni .	18·42	15·96	15·32	24·13	16·09	23·69	14·92±1·035	20·35±0·968(*a*)
Qabdi . .	Rali . .	16·29	13·84	9·85	17·34	14·40	18·17	*F=14·68	
Lasni . .	Ushni . .	14·12	No milk	13·70	20·77	15·22	21·90		

	(5) A	(6) A	(7) A	(8) A		
Percentage increase (+) or decrease (—) over first lactation	—	+10·32	—0·45	+13·24	—10·89±6·223	29·96±6·722(*b*)
	—16·83	±51·19	—12·65	+48·43	*F=19·89	
	—39·53	+25·29	—11·60	+31·29		
	—2·97	...	+7·79	...		

(*a*) Represents an increase of 36·4 per cent over two times milking.
(*b*) Represents an increase of 40·9 per cent due to increasing the frequency of milking from 2 to 4 times.
* F=Variance ratio, highly significant in both the cases.

The names of cows which were milked two times daily in the experiment are given in column 1 of Table 1. Similarly, those milked four times in the experiment are given in column 2. It will be noted that all these cows were milked only two times in their pre-experimental first lactation, the average daily yields of which are given in columns 3 and 4. A comparison of these two groups, columns 3 and 4, shows a higher average for those in column 3 than those in column 4 by 1·78 lb. Columns 5 and 7 show the second and third lactation yields under two times milking of the cows named in column 1 during the period of the experiment. ('Urji' aborted at her second calving and gave no milk.) Columns 5A and 7A indicated the percentage decreases or increases in the average milk yields in the second and third lactations respectively of cows given in column 1 over their first lactations as shown in column 3. All the cows so far considered were milked two times throughout, *i.e.*, in their first (pre-experimental), second and third (experimental) lactations.

Columns 6 and 8 show the milk yields under four times milking during the experiment in the second and third lactations respectively of the cows named in column 2. Columns 6A and 8A indicate the percentage increases in the average milk yields in the second and third lactations respectively of the cows under experimental four times milking named in column 2 over their pre-experimental first lactations under two times milking given in column 4. The percentage increases noted in columns 6A and 8A for the second and third lactations respectively are due to (i) the advancing age of the cows and (ii) increasing the frequency of milking from two to four times. Therefore, the difference

between the average values of columns 6A and 8A and of columns 5A and 7A represents the average percentage increase due only to increasing the frequency of milking from two to four times.

It will be noted from columns 6A and 8A, cows under four times milking, that increases in yield varying from 10·32 to 51·19 per cent have been obtained in the second and third lactations which may be taken as normal behaviour in milch animals advancing toward their prime or optimum age of production coupled with four times milking. Columns 5A and 7A show, however, that in the cases of animals continued on two times milking, this increase has occurred in only one case, *viz.* 'Lasni' in her third lactation and this on a lower scale than the lowest of the range of variation in the case of cows under four times milking. In all other cases there has been a distinct fall in production in the second and third lactations. In the case of 'Qabdi' this was to the extent of 39·53 per cent in the second lactation which was continued in the third to the extent of 11·60 per cent. Under two times milking it would appear that the normal development of milk secretion in Indian cows does not take place whether the optimum conditions of feed and other items of management are provided or not.

The difference between the average yields of the 'four-time milkers' and 'two-time milkers' was calculated by taking into consideration the average values of columns 6 and 8 (average yields of 'four-times milkers' in the second and third lactations respectively) and of columns 5 and 7 (average yields of 'two-time milkers' in the second and third lactations respectively).

The milk yield data of the second set of eleven cows were considered as another group for the purpose of statistical examination as shown in Table II. The same procedure of analysis was applied to this group.

TABLE II

Average milk yield (wet period) in lb. per day of eleven cows milked four times a day in their pre-experimental first lactation.

Name of cow		During pre-experimental period		During experimental period						Mean	
				2nd Lactation		3rd Lactation		4th Lactation			
Milked 2 times under experiment	Milked 4 times under experiment	1st Lactation Milked 4 times		Milked 2 times	Milked 4 times	Milked 2 times	Milked 4 times	Milked 2 times	Milked 4 times	2 times milking	4 times milking
1	2	3	4	5	6	7	8	9	10		
Poti . .	Mitri .	21·21	21·71	18·78	22·67	16·17	24·47	14·94	25·88		
Parsanni .	Machkani .	21·03	21·11	18·04	Aborted	21·62	29·64	20·43	30·72	16·44±0·883	24·79±0·945(*a*)
Rashi .	Phijji .	19·37	18·35	15·58	20·19	13·12	33·44	13·62	31·67		
Mani .	Ramdi .	18·08	18·31	16·27	19·61	12·55	18·85	15·26	21·69	*F=43·97	
Lila . .	Ruhli .	17·46	18·20	15·25	22·47	18·36	20·07	14·66	25·75		
Partapi .	..	16·76	..	15·92	..	17·83	..	18·15	..		

	5 A	6 A	7 A	8 A	9 A	10 A		
Percentage increase (+) or decrease (—) over first lactation.	—11·46	+4·42	—23·76	+12·71	—29·56	+19·21		
	—14·22	..	—0·05	+40·41	—2·85	+45·52	—12·97±1·472	27·92±5·071 (*b*)
	—19·57	+10·03	—32·27	+82·23	—29·69	+72·59		
	—10·01	+7·10	—30·59	+2·95	—15·00	+18·46	*F=36·58	
	—12·66	+23·46	+5·15	+10·27	—16·04	+41·48		
	—5·01	..	+6·38	..	+8·29	..		

(*a*) Represents in increase of 50·8 per cent over two times milking.
(*b*) Represents a decrease of 40·9 per cent due to reducing the frequency of milking from 4 to 2 times.
*F = Variance ratio, highly significant in both the cases.

The cows named in column 2 of Table II were milked four times throughout, *i.e.*, in their pre-experimental first lactations and experimental second, third and fourth lactations. It will be noted that bigger yields are recorded in the case of all these animals, except 'Machkani', who aborted at her second calving and that the increase ranges up to 82·23 per cent. It will be seen that there is an increase in each lactation averaging 19·54 lb. in first, 21·24 lb. in second, 25·29 lb. in third and 27·14 lb. in fourth lactations. Cows named in column 1, were milked four times in the pre-experimental first lactations and two times in their experimental second, third and fourth lactations. It will be noted that in the second lactation there was a distinct decrease in the case of each cow varying from 5·01 to 19·57 per cent and that the range of decrease increased with subsequent lactations. The average percentage decrease of six cows in each lactation over their first lactation being 12·16 in the second, 12·52 in the third and 14·24 in the fourth lactations. Here, the difference between the average values of columns 5A, 7A and 9A and of columns 6A, 8A and 10A represents the average percentage decrease due alone to decreasing the frequency of milking from four to two times. The increases from first to fourth lactations recorded in the case of four times milking represent the average expected behaviour of normal lactating cows. The decrease shown by the cows under two times milking represents behaviour which is at variance with what is considered normality.

As before, the difference between the average yields of the 'four-time milkers' and 'two-time milkers' was estimated from columns 6, 8 and 10 and columns 5, 7 and 9 respectively.

Finally, in Table III are given the means with their standard errors and co-efficients of variation for (*a*) total milk yield per lactation, (*b*) average milk yield per day for wet period, (*c*) average over all milk yield per day, (*d*) length of wet period, (*e*) length of dry period and (*f*) length of service period for all the cows under two times and four times milking separately.

Milk yield and live-weight graphs

Graph I shows the average milk yields of eleven 4-week periods of cows under two and four times milking in six consecutive lactations. Curve A represents the average first lactation yield of 11 cows under four times milking and Curve B represents the average first lactation yield of seven cows under two times milking. Of the 11 cows in Curve A, five went under four times milking and six under two times milking in the subsequent lactations and of the seven cows in Curve B, four were under two times milking and three under four times milking. The first lactations in both cases covered the pre-experimental period. They are both normal in type for first lactations, with the only difference, that the yields under the four times milking are on a higher level than under two times milking. A definite flush period is usually not well developed in the first lactation under either four times or two times milking. It has been our experience, working with two Indian breeds, that even in the case of good milkers there is a tendency to flatness throughout the length of the first lactation curve as compared with subsequent lactations. The daily yield at the end of the first lactation of good milkers is about half that given at the time of maximum production during the lactation.

The difference in the effects of the two systems of milking is clear from the form of the curves obtained when the subsequent yields are graphed. Four times milking shows a definite and steady upward trend with each succeeding lactation. It is the development of the flush period under the four times milking which contributes to the increases in the yields, though each lactation finishes at about the same level as the first lactation. Under the two times milking, on the other hand, no development of the flush period takes place. Although yields in the early part of the subsequent lactations are somewhat higher than those of the first lactation, the drop in yield in the course of the lactation is sudden, and this rapid downward trend results in daily yields below those of the first lactation and a lower final total lactation yield. There is also a tendency to a shortening of the lactation period. Regardless as to whether the cows were milked four times or two times in the first lactation, there was a definite fall under two times milking in the subsequent lactations and similarly there was an increase in each subsequent lactation under four times milking.

TABLE III

Mean and coefficient of variation for lactation yield, wet average, overall average wet period, dry period and service period

Particulars of treatments and lactations	Lactation yield			Average yield per day for wet period			Average overall yield per day			Length of wet period			Length of dry period			Length of service period		
	N*	Mean±S.E. (lb)	C.V.	N	Mean±S.E. (lb)	C.V.	N	Mean±S.E. (lb)	C.V.	N	Mean±S.E. (days)	C.V.	N	Mean±S.E. (days)	C.V.	N	Mean±S.E. (days)	C.V.
Cows milked two times																		
2nd Lactation . .	9	3791·4±272·8	21·6	9	15·4±0·86	16·7	9	8·3±0·59	21·2	9	240·3±16·65	20·0	9	220·3±47·44	64·6	9	182·2±42·84	70·5
3rd ,, . .	10	4450·4±388·5	27·6	10	16·5±0·89	17·0	9	11·1±1·10	29·7	10	268·3±13·79	16·3	9	134·9±20·80	46·3	8	115·6±7·62	18·6
4th ,, . .	8	4081·5±389·9	27·0	8	16·4±0·81	13·9	8	10·9±1·12	29·2	8	248·8±18·92	21·5	8	129·4±21·36	46·7	8	95·6±8·51	25·2
5th ,, . .	2	4306·5±712·5	23·4	2	16·5±2·05	17·5	2	10·8±1·37	17·8	2	259·0±11·00	6·0	2	136·0±5·00	5·2	2	113·0±15·00	18·8
6th ,, . .	2	2096·0±28·0	1·32	2	13·4±0·26	2·1	1	8·2±0·00	0·0	2	223·5±5·50	3·5	1	140·0±0·00	0·0	2	97·50±13·50	19·6
All lact. taken together	31	4060·8±188·5	25·8	31	15·9±0·45	15·7	29	10·1±0·54	28·7	31	254·3±8·15	17·9	29	160·1±18·11	60·9	29	129·3 ±14·83	61·7
										Average calving interval=414·4 days								
Cows milked four times																		
2nd Lactation . .	8	6155·1±294·9	13·6	8	20·7±0·82	11·3	8	13·6±0·89	18·6	8	297·3±4·49	4·3	8	162·8±25·31	44·0	8	178·1±24·05	39·3
3rd ,, . .	9	7092·4±541·2	22·9	9	23·2±1·77	22·8	9	16·3±1·68	30·8	9	305·9±0·11	0·1	9	143·6±24·72	51·7	9	157·6±23·17	44·1
4th ,, . .	7	7861·1±533·7	18·0	7	26·2±1·60	16·2	7	19·6±1·12	15·2	7	299·6±4·39	3·9	7	101·4±6·22	16·2	7	113·1±6·77	15·8
5th ,, . .	5	7641·0±654·1	19·1	5	25·0±2·12	19·0	5	15·9±1·15	16·2	5	305·4±0·60	0·4	5	185·4±57·01	69·8	5	139·4±25·82	44·3
6th ,, . .	2	6369·0±113·0	2·5	2	20·8±0·37	2·5	2	16·0±0·20	1·8	2	306·0±0·00	0·0	2	93·0±12·00	18·5	2	109·0±0·00	11·7
7th ,, . .	1	6786·0±0·0	0·0	1	22·2±0·00	0·0	1	16·2±0·00	0·0	1	306·0±0·00	0·0	1	48·0±0·00	0·0	1	71·0±0·00	0·0
All lact. taken together	32	7056·9±246·8	19·8	32	23·3±0·78	18·9	32	16·4±0·69	23·8	32	302·3±1·56	2·9	32	139·5±13·72	55·6	32	141·8±10·54	42·0
										Average calving interval=441·8 days								
Percentage increase over two times milking	74			47			61											

*N = Number of lactations averaged

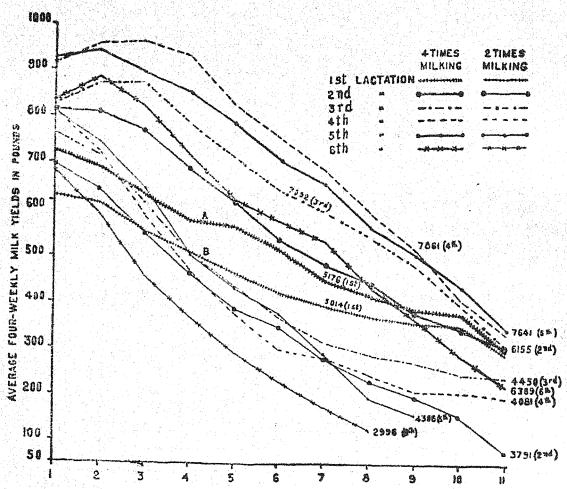

FIG. 1. Average milk yields of cows under two and four times milking 4 weekly periods.

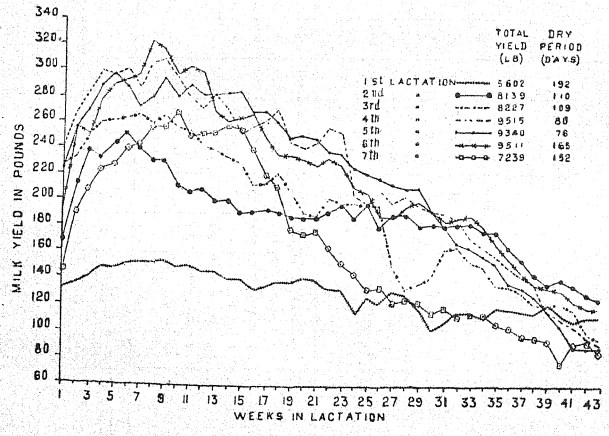

FIG. 2. Lactation curves of Sahiwal cow "Narace" under four times milking.

From the experience of four times milking on two different Indian breeds (Sahiwal and Tharparkar), it has been found that cows with normal first lactations under two times milking when put under four times milking in subsequent lactations, develop milk capacity in a manner similar to those which have been under four times milking from first calving. The shape of the graph and the yield during the last three months of the first lactation is a good index to subsequent yields.

FIG 2.

It will be of interest to compare these milk yield curves with those of a typical cow of the Sahiwal breed under four times milking. Graph II shows the yields of the Sahiwal cow 'Naraee' under four times milking in seven consecutive lactations. The first lactation curve shows the typical flatness indicating high possibility of persistency in yields as demonstrated in the subsequent lactations. There has been normal development of flush period in each subsequent lactation up to the sixth, the seventh lactation showing the beginning of the decline with age.

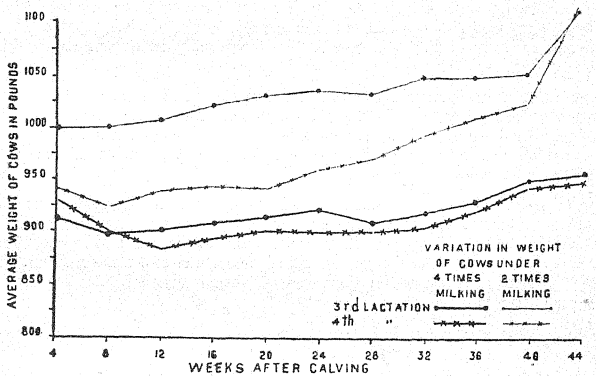

FIG. 3. Average weight of cows under two and four times milking.

When the yield curves of cows under two and four times milking are considered along with curves showing the variation in weight during the lactation, as in Graph III, it is seen that under two times milking cows lost little weight after calving, and made speedy gains throughout the lactation, although the concentrates given in both the cases were in proportion to the milk yields. There is a definite fall in weight of those under four times milking coinciding with the flush period and this loss may amount to 100 lb. or more which is normal to cows of high milk yield.

DISCUSSION

The percentage increases of the second and third lactations over the first lactation of cows under two times milking in the first lactation and four times milking in the subsequent lactations are compared with those of the cows which were retained on two times milking in the second and third lactations (*vide* Table I). There is an increase of 41 per cent in yield which is due alone to increasing the frequency of milking from two to four times. The actual difference between the average yields of the 'four-time milkers' and 'two-time milkers' in their second and third lactations in this group shows an increase of 36 per cent of milk yield in favour of the four-time milking. Similarly, when the percentage increases of the second, third and fourth lactations over the first lactation of cows under four times milking in the first lactation and continued on four times milking in the subsequent lactations are compared with the percentage decreases in the second, third and fourth lactations over the first lactation of cows under four times milking in the first lactation and two times milking

in the subsequent lactations (*vide* Table II), a decrease of 41 per cent of yield is observed which is attributed to reducing the frequency of milking from four to two times, since all other conditions so far as these are controlable were the same. The actual difference between the average yields of the 'four-time milkers' and 'two-time milkers' in their second, third and fourth lactations is 51 per cent. All these increases and decreases are statistically significant. Taking all cows under two times milking and four times milking separately the percentage increases due to four times milking are 74 per cent for lactation yield, 47 per cent for average daily milk yield and 61 per cent for average overall daily milk yield. The lower percentage for daily yield as compared with lactation yield is due to the effect two-time milking has in decreasing the number of days in milk. These striking increases due to four times milking coupled with 'handling' are far in excess of results obtained with other breeds abroad. Ragsdale *et al.* [1934] after experimenting with Jersey and Ayrshire cows in Missouri (U. S. A.) found that four times milking effected 16 per cent increase of milk over cows milked twice a day. In Maryland (U. S. A.) with Holsteins, Woodward [1931] found that three times milking gave 20 per cent more milk and 21 per cent more butter-fat than cows milked twice a day. Campbell [1930] found that the average increased yield of nine Graded Shorthorns (at the Reading University Farm, U. K.) in their thrice daily milking year over the yield in their preceding lactation was 19·3 per cent.

The average calving interval for cows under four times milking is 442 days, which is about 28 days longer than that of cows under two times milking. This is due to the slightly longer service period necessitated by longer lactation period in the case of cows under four times milking. But this longer calving interval has not affected the overall average, which is 61 per cent more than in the case of the cows under two times milking.

Table IV shows the trend of yields of cows in the Sahiwal and Tharparkar herds in five successive lactations under two and four times milking. It will be seen that under four times milking, the first lactation yield is 55 per cent higher in the case of the Tharparkar and 70 per cent higher in the case of the Sahiwal than the corresponding yield under two times milking. Yet under four times milking, most of the later lactations show significant increase over the first lactation which is not the case under two times milking.

TABLE IV

Average lactation yields under two and four times milking in the Tharparkar and Sahiwal herds

Two times milking				Four times milking			
Lact. No.	No. of cows.	Mean ± S.E. (lb.)	C.V.	Lact. No.	No. of cows.	Mean ± S.E. (lb.)	C.V.
	(Before 1937)		Tharparkar herd (Karnal)			(After 1937)	
1	61	3483±191	42·8	1	109	5403±158	30·5
2	61	3365±178	41·3	2	109	5540±175	33·0
3	48	3834±219	39·6	3	55	6235±234	27·8
4	37	3884±247	38·7	4	33	6176±341	31·7
5	24	3708±334	44·2	5	10	5987±822	43·4
	(Before 1932)		Sahiwal herd (Delhi)			(After 1932)	
1	99	2944±120	40·4	1	50	5008±210	29·6
2	89	3091±126	38·5	2	36	5612±279	29·9
3	77	3367±130	33·9	3	18	6174±402	27·7
4	59	3514±153	33·4	4	11	6373±498	25·9
5	44	3128±187	39·7	5	5	6129±391	14·3

The results obtained in the experiment are thus in line with practical experience with the Sahiwal herd at both Pusa and New Delhi and with the Tharparkar herd at Karnal. In Table V are given the records of the two herds which clearly indicate the improvement effected by the introduction of four times milking and 'handling' since 1932-33 in the case of Sahiwal herd and 1936-37 in the Tharparkar herd.

TABLE V

Records of the Sahiwal and Tharparkar herds under four times milking

Year (July to June)	Sahiwal herd				Tharparkar herd			
	Wet average in lb. per day	Overall average in lb. per day	Total number of cows (in milk and dry) per day	Percentage of cows in milk per day	Wet average in lb. per day	Overall average in lb. per day	Total number of cows (in milk and dry) per day	Percentage of cows in milk per day
1931-32	*13·6*	*6·9*	*79*	*50·2*				
1932-33	17·9	9·9	77	55·1				
1933-34	18·7	12·1	63	66·2				
1934-35	19·1	11·5	54	60·2				
1935-36	21·2	10·9	58	51·5	*14·4*	*9·3*	*95*	*56·0*
1936-37	20·7	9·3	71	44·9	17·4	9·5	91	54·0
1937-38	22·2	13·2	73	59·8	20·4	11·4	89	56·0
1938-39	21·2	13·0	65	61·4	19·2	11·1	97	55·0
1939-40	21·9	14·9	70	67·8	19·6	11·3	82	58·1
1940-41	21·8	15·3	72	70·2	19·5	11·7	88	60·1
1941-42	20·0	11·7	88	59·4	20·1	15·5	89	76·3
1942-43	19·7	13·3	79	67·4	22·2	14·3	89	76·0
1943-44	21·6	15·5	82	72·5	21·4	16·2	87	5·0
1944-45	20·4	14·9	92	72·9	20·2	13·6	95	66·6

NOTE.—Figures in italics indicate records under two times milking.

There is no doubt that two times milking is too infrequent for these breeds and induces a tendency to develop flesh instead of milk production. By more frequent milking on the other hand the processes of milk stimulation are increased and food is converted into milk rather than into flesh. The animals develop a more active and alert disposition which approaches, what in dairy literature is generally referred to as 'milk temperament'. Handling and prenatal milking possibly also contribute something to the same result. The practice conduces to a healthy and active udder condition; congestion after calving is practically negligible, milk fever and mastitis unknown. The absence of these troubles which are the cause of much anxiety in dairy herds in all western countries has elicited much favourable comment from overseas visitors during the war who have been impressed by the results.

The extra cost involved in four times milking as against two times milking is represented merely by the cost on account of wages of extra labour plus the cost of extra concentrate ration required by the cow to produce the extra milk. The amount of roughage required for maintenance will remain the same. In this experiment, the overall yield under two times milking was about 10 lb. per head per day and that under four times milking about 16 lb. per head per day. Assuming that one attendant can milk eight cows twice daily and that an extra attendant is required for four times milking, the extra milk, 6 lb. per head per day or $8 \times 6 = 48$ lb., of the eight cows will require 24 lb. extra grain which at Rs. 8 a maund (82·3 lb. present price) will cost Rs. 2-6. This together with the wages of one extra attendant at Rs. 1-8 per head will cost Rs. 3-14. Against this sum, the value of 48 lb. of extra milk at 8 lb. per rupee is Rs. 6. Thus taking the overall period from calving to

calving, which in these experiments averaged 442 days, the net gain due to four times milking on eight Tharparkar cows is Rs. 2-2 per day.

There can be no doubt that the practice of two times milking with many Indian breeds results in the country having to maintain a larger number of animals than is necessary for the production of the same amount of milk. The experimental results referred to suggest that this is not less than 47 per cent and that probably 61 per cent is a nearer figure for the Tharparkar cows under the experiment. In a country where labour is not an expensive item there is here a case of increased reward out of proportion to the cost of labour involved.

There has been no decrease in the fat percentage in milk which has remained at about five per cent in both the herds since the introduction of four times milking.

Experience in the general management of the herds in question suggests that three times milking has an effect similar in manner, if not to the same degree, as four times milking.

Summary

At the Agricultural Substation, Karnal, 11 Tharparkar cows were milked four times in a day and 'handled' *i.e.*, udder massaged 15 to 20 days before calving and milked as secretion started, while 12 cows were milked twice a day and not 'handled'.

The increase in the average yield per day for the wet period due to four times milking and 'handling' was 47 per cent over cows milked twice a day.

The overall average and lactation yield showed 61 per cent and 74 per cent increases respectively.

The calving interval for cows under four times milking was 442 days and that for cows under two times milking was 414 days. This variation was due to the slightly longer service period necessitated by the longer lactation period in the case of cows under four times milking. But this longer interval has not affected the overall average which was 61 per cent more than the cows milked twice daily.

When the yields of cows under the two and four times milking were graphed, the latter showed a definite upward trend of the curves with each succeeding lactation and a development of flush period. Under the two times milking no development of the flush period took place, and the yield curves showed a rapid downward trend in the later lactations.

Acknowledgements

Our thanks are due to Messrs. N. P. Fernandez and S. Zahir-ud-din for their painstaking supervision of the experiment and accurate recording of the results during their respective terms of office as Cattle Superintendents at Karnal. Special thanks are due to Mr Wynne Sayer who, as Imperial Agriculturist, introduced many new methods into the technique of management in Indian breeds of dairy cattle. The soundness of his methods has been fully demonstrated in the successful development of the Sahiwal and Tharparkar herds, in the latter of which, the experiment was initiated, by the Imperial Council of Agricultural Research, to verify by actual test his claim that Indian breeds of dairy cattle require milking more than two times daily in order to stimulate and develop their full capacity for milk yield.

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THE EFFECT OF CALCIUM SUPPLEMENT ON THE DIGESTION OF ORGANIC NUTRIENTS IN FODDERS IN WHICH LIME CONTENT IS EITHER LOW OR HAS A TENDENCY OF POOR ASSIMILATION

By RAMESWAR MUKHERJEE, M.Sc., Late Assistant Physiological Chemist and INDUBHUSAN CHATTERJEE, M.Sc. (AGR.), L. Ag., Formerly Physiological Chemist, Department of Agriculture, Bengal

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THE study of the possible effect of a deficiency or deprivation of mineral elements on the digestibility and utilization of organic nutrients of feed does not seem to have received much attention. Ranganathan and Rao [1938] studied the influence of calcium intake and found that an increase in the ingestion of calcium of rats improved both the digestibility and biological value of protein. In similar experiment Swaminathan [1931] did not observe any effect of calcium intake on the biological value of protein. Henry *et al* [1940] working on rats found the same value for the biological value and true digestibility at two widely different levels of calcium ingestion, viz. 0·07 per cent and 0·86 per cent calcium. Earlier work of Woodman and Evans [1930] supports the finding of the latter worker, and their results on sheeps showed that the digestibility of organic nutrients was not affected by the deficiency of minerals (specially calcium and phosphorous) in the feed.

The investigation forming the subject matter of this paper arose from the following consideration. A feeding and metabolic test was initiated with Napier silage which was however found to be very poor in lime (0·29 per cent CaO on dry basis and 0·07 per cent on fresh basis). It should be noted that Napier grass is the recommended fodder from Bengal Agricultural Department and silage was made from this grass. The experiment was therefore designed with a view to see amongst others how far the addition of calcium supplement given as $CaCO_3$ was likely to be reflected on the digestibility of organic nutrients. This represented an experiment in which the fodder was admittedly deficient in lime.

It has been found in a series of previous trials that the lime present in rice straw has a tendency of poor assimilation. An experiment with Boro variety of rice straw with and without lime given as $CaCO_3$ provided the material for studying their effect on the digestibility of the same nutrients.

EXPERIMENTAL

Both under Napier silage as well as Boro straw eight bullocks of approximately same age and live weight were selected four being placed under 'No lime' and four under 'Lime'. The animals under 'Lime' were each given 25 gm. of $CaCO_3$ daily.

The Napier Silage was made at Dacca Farm silo pit and was fed *ad lib.* to all the eight animals. Linseed cake at the rate of 0·5 gm. per lb. of live weight was fed as concentrate to the silage animals. The animals were watered twice daily. The feeding lasted altogether for 50 days and the metabolic collection commenced after 29 days and continued for 13 days.

In the case of Boro straw also it was fed *ad lib.* The concentrate given was mustard cake at the rate of 10 per cent on the straw actually consumed. The feeding lasted for a total period of 83 days and the metabolic collection commenced after a preparatory feeding of 59 days and continued for 10 days.

In both the groups common salt was given at the rate of 25 to 40 gm. per animal per day.

RESULTS AND DISCUSSIONS

It should be noted that both these experiments have been recorded in the *Annual Reports* of the Physiological Chemist, Bengal, for the years 1936-37 and 1938-39. A paper on the nutritive value of Boro straw also forms the subject matter of a separate communication by Chatterjee and Sarkar [1947].

Here the relevant data in reference to the digestibilities as affected by 'Lime' and 'No lime' under both Napier silage and Boro straw, are assembled in Tables I-IV.

TABLE I

Comparative figures on combined digestibilities and calcium metabolism. (Calcium data computed on 500 lb. live weight)

Particulars		No lime					Lime				
		D1	D2	D6	D8	Mean	D3	D4	D7	D9	Mean
		Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Digestibility co-efficients of combined feed, viz. (silage and cake)	Dry matter	41·0	43·5	41·2	35·5	40·8	41·5	40·6	41·8	41·2	41·3
	Organic matter	43·6	46·2	43·8	38·6	43·0	45·0	43·7	45·2	44·6	44·6
	Crude Protein	36·1	40·4	40·7	31·4	37·1	36·9	37·8	35·7	37·8	37·1
	True protein	38·6	42·6	49·5	38·0	42·2	42·8	38·9	39·5	39·4	40·2
	Crude fibre	47·3	50·4	46·6	44·1	47·1	47·8	50·8	51·8	50·0	50·0
	N. F. extract	40·7	42·7	41·4	34·0	39·7	43·1	37·6	40·7	40·2	40·4
		gm.	gm.	gm.	gm.		gm.	gm.	gm.	gm.	
Metabolism of lime (computed on 500 lb. live weight)	CaO from silage	8·64	7·47	7·37	8·04		8·56	7·54	7·60	8·19	
	CaO from cake	1·51	1·50	1·49	1·48		1·49	1·50	1·47	1·48	
	CaO from salt	0·02	0·01	0·02	0·01		0·01	0·02	0·01	0·01	
	CaO from water	0·11	0·11	0·10	0·12		0·07	0·11	0·12	0·12	
	Lime supplement	..	..	..	..		9·83	9·08	10·55	9·97	
	TOTAL	10·28	9·09	8·98	9·65		19·96	18·25	19·75	19·77	
	CaO form faeces	11·96	11·13	10·88	12·30		20·77	18·51	18·84	19·35	
	Difference	−1·68	−2·04	−1·90	−2·65		−0·81	−0·26	0·91	0·42	
	Urine	0·12	0·27	0·16	0·14		0·05	0·13	0·11	0·11	
	Balance	−1·80	−2·31	−2·06	−2·79		−0·84	−0·39	+0·80	+0·31	

At the outset it is necessary to state that so far as the consumption of Napier silage was concerned the animals under both 'No lime' and 'Lime' ate the roughage more or less in the same proportion. The concentrate given was according to live weight. The main difference was in the addition or otherwise of the lime supplement. It will be seen from Table I above that the addition of lime has definitely contributed towards a better calcium assimilation, there being two clear positive balances, one, *viz.*, D4, is on the border line (it is only negative by a small margin) while the remaining one is slightly more negative but still distinctly better than any of the four under 'No lime' group. In the latter group the negative values in all have been numerically much larger. There is therefore no doubt of the better effect of lime supplement on the efficiency of lime assimilation.

If now the digestibility values are examined in regard to the bearing of lime assimilation, it will be noted that, in so far as can be seen from the digestibilities of combined feeds, the difference with respect to the majority of components is negligible. A slight depression in the digestibility of ether-extract and also a slight increase in that of crude fibre, both under 'Lime', are only partially suggested from the individual as well as mean values. The mean value of true protein is also slightly low under 'Lime' but this has been chiefly due to the fact that under 'No lime' one individual value, *viz.* of D6, has been much more. This has correspondingly increased the mean under this group. But for this, there is no significant difference in the digestibility of either crude protein or true protein. In the case of ether-extract or crude fibre, the difference appears to be more marked. This is also noticed when from the combined values the corresponding shares of cake are deducted as can be seen from Table II.

TABLE II

Digestible co-efficients of ether-extract and crude fibre in Napier silage. (Obtained by the method of elimination)

Group	Animals No.	*Ether-extract*	*Crude fibre*
"No lime"	D1	35·22	47·94
	D2	33·88	51·19
	D6	20·72	47·33
	D8	29·82	44·71
Mean		29·91	47·79
"Lime"	D3	26·39	48·49
	D4	25·11	51·63
	D7	18·13	52·10
	D9	24·54	50·69
Mean		23·54	50·73

It should be noted that the difference here is more pronounced than in the case of combined feed.

It will be interesting at this stage to examine the values obtained with Boro straw. These are assembled in Tables III and IV.

TABLE III

Boro straw mustard cake. Comparative figure on the digestibilities of combined feed and the nature of calcium metabolism under "No lime" and "Lime"

		"No lime"					"Lime"				
		D3	D4	D7	D9	Mean	D1	D2	D6	D8	Mean
		Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Coefficients of digestibilities of combined feeds (straw and cake)	Dry matter	44·03	40·95	42·17	43·42	43·39	41·25	43·16	41·17	39·49	41·34
	Organic matter	52·85	54·09	52·68	52·68	53·07	51·04	49·97	50·30	48·73	17·76
	Crude protein	55·40	53·66	53·86	53·10	53·00	52·47	52·68	54·72	55·29	53·04
	True protein	50·19	46·48	42·97	48·41	47·01	45·77	48·15	47·08	44·67	46·42
	Ether-extract	58·92	55·03	56·63	57·68	56·56	53·85	36·84	45·22	50·16	48·72
	Crude fibre	62·45	62·21	61·46	63·14	62·31	60·37	45·87	56·81	57·87	55·23
	N. F. extract	45·77	48·78	46·45	45·47	46·62	44·45	52·61	45·23	40·79	45·77
		gm.	gm.	gm.	gm.		gm.	gm.	gm.	gm.	
Metabolism of lime (CaO) computed on 500 lb. live weight	CaO from straw	13·65	12·82	13·93	13·46		12·65	11·74	14·08	13·81	
	CaO from cake	4·53	4·33	4·85	4·55		4·35	4·03	4·82	4·75	
	CaO from water	0·08	0·09	0·09	0·10		0·09	0·08	0·11	0·09	
	CaO from salt	0·10	0·10	0·12	0·11		0·11	0·10	0·10	0·12	
	CaO added	..	..	..	..		9·66	9·16	9·05	10·68	
	TOTAL	18·36	17·34	18·98	18·22		26·86	25·11	28·16	29·45	
	Faeces	20·53	19·14	21·88	20·46		27·96	22·84	30·19	30·40	
	Difference	—2·17	—1·80	—2·90	—2·25		—1·10	2·27	—2·03	—1·03	
	Urine	0·73	1·11	1·10	0·78		1·00	1·65	1·79	0·99	
	Balance	—2·90	—2·91	—4·00	3·03		—2·10	+0·12	—·82	—2·02	

TABLE IV

Mean digestibility of Boro straw (Obtained by the method of elimination)

Particulars	No. Lime	Lime
Dry Matter	41·27	39·00
Organic matter	51·33	47·89
Crude protein	44·53	44·07
True protein	31·74	30·61
Ether-extract	32·55	16·52
Crude fibre	63·71	56·45
N. F. extract	45·86	44·93

The results of Tables III and IV first require to be compared amongst themselves and then with those of Tables I and II. Judged, whether on the basis of combined digestibilities (Table III) or on the digestibilities of straw components (Table IV), lime feeding has definitely depressed the digestibility of organic matter mainly from the share of ether-extract and crude fibre.

It should be noted also that unlike under Napier silage (Table I) the addition of lime supplement under Boro has not contributed in any way towards a better lime assimilation ; but the effect has been well reflected in depressing the digestibility of both ether-extract and crude fibre.

In so far as ether extract is concerned,—lime feeding has been followed by a lowered digestibility; under both Napier grass and Boro straw. This is also in conformity with what might be expected since the presence of lime offers the possibility of formation of insoluble calcium soap which eliminates out with the faces as undigested matter thereby reducing the quota of digested share.

In the case of fibre however the caustic action of lime is likely to reduce the resistance of the tough integuments of the fibre thereby helping in a better digestion. This has happened also with Napier silage, but not only has it failed under Boro straw with lime but there has even been a depression in its digestibility. This is difficult to explain.

Here it is worthy of note, as already stated, that under Napier silage the balance under lime feeding has been either positive or very slightly negative. On the other hand Boro straw has exhibited a negative lime balance which is almost identical under both 'Lime' and 'No lime'. The only other difference between them is that Napier silage was fed with what was supplied as linseed cake, whereas Boro straw was fed with mustard cake. But the main reason for which lime assimilation in Boro straw has been very poor, is due to the fact that this straw contains a very large percentage of oxalic acid.

SUMMARY

Two feeds of which one (*viz.* Napier silage) was deficient in lime and the other (*viz.* Boro rice straw) had a tendency of poor lime assimilation, were fed to bullocks with and without supplement of calcium as $CaCO_3$. It was found that amongst the organic nutrients ether-extract was most susceptible to a lowered digestibility. The fibre fraction was also found to be affected but the results were not identical since under Napier feeding the addition of lime supplement effected an increase in its digestibility, but under Boro straw it was lowered. It may be stated that in the case of Napier silage the addition of lime definitely contributed towards a better lime assimilation and fibre has been better digested under 'Lime' combination. If this is ascribed to the better effect of lime, there is no reason why it should have been different under Boro straw feeding under similar combination with lime. In the case of ether extract the presence of lime offers the possibility of the formation calcium soap thus holding out lime. This might account for the depressed digestibility found in the ether-extracts of both Napier silage and Boro straw, but we have at present no explanation as to why the fibre digestibility under silmilar lime combination was increased under Napier feeding and lowered and Boro straw feeding.

It is also necessary to state that the poor lime assimilation under Boro straw feeding is associated with a large percentage of oxalic acid in it.

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THE VALUE OF BORO RICE STRAW AS A CATTLE FEED

INDUBHUSHAN CHATTERJEE, M. Sc. (AGR.), L. AG., Formerly Physiological Chemist, Department of Agriculture, Bengal, and SACHINDRA NATH SARKAR, M. Sc., Ph. D.

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IN Bengal the rice crop is known by Amon, Aus and Boro. The Amon on winter variety is harvested during winter (November to January) and the Aus variety is harvested in August-September. The Boro variety is generally a summer or spring crop. But a second or what may be called *kharif* crop, is also taken in some places. The summer variety is sown in seed bed in October or November, transplanted in December-January and harvested in March to June. The *kharif* variety is sown in seed bed in June or July, transplanted in July-August and harvested in September-October. The crop is grown by artificial irrigation in low lying lands adjoining beels, streamlets and rivers.

The total area of Boro crop in Bengal is a little over 400,000 acres being about 1·75 per cent of the total rice area of the province. Of this the district of Mymensing occupies nearly half followed by Dacca (about 45,000 acres), Malda 40,000 acres, Tiperah 30,000 acres, Khulna 25,000 acres, Midnapur 17,400 acres and Rajshahi 13,500 acres. In the other districts it varies from 300 to 8,000 acres whereas the districts of Birbhum, Jalpaigury and Chittagong hill tract have no Boro area.

Judged from the standpoint of the acreage it is a comparatively minor crop and is mainly distributed in the eastern districts, but neither the grain nor the straw can be a matter of neglect under the existing shortage of our food and fodder.

The straw obtained from this crop is generally liked by animals and as will appear from the investigation on it, it compares well with other straws in its feeding values. As this crop is harvested at a time when the monsoon does not permit efficient drying, the straw suffers from somewhat greater disadvantage than that from Aus crop. There cannot be any doubt that every year a large percentage of both these straws have to pay a heavy toll to the vagaries of monsoon. Yet a considerable part of it can be saved if suitable ensiling could be adopted. Here of course great propaganda is needed to impress it on the minds of the people and also to overcome their inherent disinclination and inertia.

Turning how to the experiment, the Boro straw was obtained from the riverine tracts of the Mirpur side of the Dacca district. About 200 maunds were obtained and feeding was commenced from the 30 June, 1936 and terminated on the 23 September of the same year thus lasting for about 12 weeks.

Before describing the details of the experiment it may be well to examine the composition of Boro straw side by side with that of the other straws. This is given in Table I.

It will be noted from Table I that the straw contains a much higher percentage of nitrogen as compared to that of Amon or Aus. It has recorded the lowest percentage of crude fibre as well as nitrogen-free extract. This has been mainly due to the fact that its mineral content (ash) is very high (Nearly 22 per cent) as compared to 11 to 16 per cent in the case of the other straws. The striking feature is that the major part of this ash consists of acid insoluble residue (over 18 per cent) so that the actual soluble matter is about the same (3·8 per cent) as in others. It is poorer in lime than both Amon and Aus, but the potash content is similar. As will be seen later it contains an appreciable quantity of oxalic acid.

Previous experiments have shown that the lime assimilation under rice straw feeding from the share of lime present in the straw is generally low. It was therefore decided to divide the feeding and digestion trails under two heads, *viz.* (1) with no lime and (2) with lime supplement as $CaCO_3$. The latter was given mixed with cake at the rate of 25 gm. to each animals. Mustard cake was fed as concentrate on the basis of 10 per cent on the wieght of straw consumed.

In view of the necessity of economising space it is not possible to give the various data ; but may be stated that both under 'No lime' and 'Lime' groups the animals showed a definite increase in live weight. The consumption of dry matter also recorded a steady increase. As the feeding proceeded there was somewhat greater demand of water. The nature of water consumption and urination did not exhibit any difference between either.

TABLE I

Composition of Boro and other straws

Particulars	Boro straw	Aus straw			Amon Straw		
		Dacca		Krishnagar	Dacca	Krishnagar	Rangpur
	47 1936	117 1933	62 1939	20 1936	15 1934	138 1934	10 1936
Organic matter	78·060	88·796	85·528	83·868	86·786	86·180	83·571
Crude protein	6·344	5·879	3·850	3·788	3·750	3·063	3·806
True protein	5·034	5·174	3·319	3·625	3·163	2·844	2·400
Ether extract	0·936	1·713	1·222	1·276	1·147	0·800	1·15
Crude fibre	22·069	32·425	32·940	33·899	33·239	32·600	34·137
Nitrogen-free extract	41·712	48·779	47·516	44·905	48·650	49·717	44·477
Ash	21·940	11·204	14·472	16·132	13·214	13·820	16·429
Insoluble ash	18·129	7·372	11·384	11·552	9·082	10·120	12·695
Soluble ash	3·811	3·832	3·088	4·580	4·132	3·700	3·734
Lime	0·343	0·635	0·644	0·561	0·500	0·480	0·399
Magnesia (MgO)	0·297	0·400	0·316	0·310	0·365	0·220	0·475
Potash (K_2O)	1·856	2·032	1·030	2·466	1·838	2·040	1·622
Soda (Na0)	0·085	0·207	0·234	0·788	0·261	..	0·024
Phosphate (P_2O_5)	0·287	0·176	0·234	0·437	0·108	0·06	0·163
Sulphate (SC_4)	0·523	0·182	0·288	0·280	..	0·280	0·129
Chloride (Cl_2)	0·464	0·336	0·199	0·098	0·406	0·90	0·339

The mean digestibility and co-efficients under 'No lime' and "Lime" groups are given in Table II. These have been worked out by the method of elimination.

TABLE II

Digestibility co-efficients

Components	Boro straw		Aus straw	Amon straw
	'No. lime'	'Lime'		
Dry matter	41·27	39·00	43·90	45·60
Organic matter	51·33	47·89	47·40	51·03
Crude protein	44·53	44·07	30·00	9·29
True protein	31·74	30·61	25·04	12·10
Ether-extract	32·55	16·52	28·00	43·80
Crude fibre	63·71	56·45	58·90	61·90
Nitrogen free extract	45·86	44·93	43·30	46·40

It will be seen from above that the feeding of lime as calcium carbonate did not give any better results. The digestibilities of dry matter and organic matter have been slightly but definitely depressed. It has been still more so in the case of ether-extract and crude fibre. Moreover ether-extract has exhibited great fluctuation specially under individual values (not shown in Table II) in which one animal even recorded negative digestibility, another about one third to one-fourth of the same under 'No lime' group while still another gave very high value. The results under 'No lime' have been of a more stable nature.

The total digestible nutrients per 100 lb. of straw under both groups are set up in Table III.

TABLE III

Total digestible nutrients per 00 lb. of straw (dry basis)

Particulars	Boro straw		Amon straw	Aus straw
	'No lime'	'Lime'		
Total digestible nutrients	41·14	38·28	44·13	43·02
Digestible crude protein	2·83	2·80	0·39	1·77
Starch equivalent	24·03	21·21	24·60	23·97
Nutritive ratio	1:13·40	1:12·70	1:1·13	1·23

In the above the corresponding values of Amon and Aus straw have also been shown. The most noteworthy feature is that, compared to the other straws it possesses the highest amount of digestible protein which is necessarily reflected in a narrower nutritive ratio which approximates closely to the maintenance requirements. Another point of interest is that although in terms of T. D. N. it has recorded a lower value than both Amon and Aus, its net energy value (starch equivalent) is almost the same. This is mainly due to its low fibre content.

It will be interesting at this stage to compare the results on the basis of the performance of the animals per say 1,000 lb. live weight. These are set up in Table IV.

TABLE IV

Comparative performance of animals under different straws per 1,000 lb. live weight

Paticulars	Boro		Amon		Aus	
	10 per cent cake "No lime"	mustard Dacca "Lime'	Dacca Linseed cake	Krishnagar mustard cake	Daca lb. cake	Krishnagar mustard cake
	lb.	lb.	lb.	lb.	lb.	lb.
Consumption of feed—						
Straw	15·27	15·06	13·62	11·72	14·95	14·56
Cake	1·49	1·49	1·40	1·16	1·04	1·45
Total	16·76	16·55	15·02	12·88	15·99	16·02
Total digestible nutrients	7·24	6·73	7·08	6·56	7·31	8·78
Crude protein	0·84	0·83	0·43	0·44	0·52	0·46
Protein equivalent	0·71	0·70	0·42	0·31	0·46	0·37
Ether extract	0·16	0·14	0·18	0·18	0·14	0·20
Crude fibre	2·83	2·49	2·86	2·81	2·65	4·29
Starch equivalent	4·62	4·14	4·34	4·18	4·23	5·43
Nutritive ratio	1:7·5 to 7·9	1:6·8 to 7·5	1:13·4 to 17·5	1:17 to 19	1:12 to 13	1:13 to 17

The main features however are that compared to other straws the consumption of Boro straw is definitely higher but in spite of it the nutritive ratio is narrowest (6·8 to 7·7) on account of higher availability of digestible protein. This is a particular advantage in its favour, as it means that when this straw is fed the amount of concentrate can be correspondingly reduced. As a matter of fact

the nutritive ratio given in Table III rather suggests that for the purpose of maintenance, concentrates can be completely dispensed with. This is however not advisable as it has been found in other experiments that when rice straw is fed without concentrate it adversely affects economic utilization. But the quantity can be safely reduced to half or possibly even less. A careful feeder can adjust it after watching the effect on the animal for a short period.

Nitrogen and Mineral Metabolism.

As all the figures connected with above will occupy large space only the digested share in the case of nitrogen and total intakes in the case of minerals are set up in Table V.

Table V

Digested nitrogen and total intake of minerals

Particulars	Animal	Live weight	Digested nitrogen	Computed on 500 lb. live weight					
				CaO	Mg.	K_2O	Na_2O	P_2O_5	Cl
No lime Straw and Mustard cake	D3	753	55·40—	13·65—	11·82+	73·86—	3·38+	11·42+	18·47—
	D4	778	55·52+	12·82—	11·10+	69·36—	3·18+	10·73—	17·34—
	D7	661	47·30+	13·93—	12·06+	75·35—	3·45+	11·65—	18·84—
	D9	712	48·04+	13·46	11·65+	72·81—	3·34+	11·26+	18·20—
Lime straw and Mustard Cake	D1	717	45·24+	12·65—	10·95+	68·45—	3·14+	10·59+	17·11—
	D2	757	44·47+	11·74+	10·17+	63·50—	2·91+	9·82+	15·88—
	D5	766	55·97+	14·08—	12·19+	76·17—	3·49—	11·78—	19·04—
	D6	649	47·12+	13·81—	11·96+	74·74—	3·42+	11·56—	18·68—

*The symbols + and — indicate whether the balance was positive or negative

In Table V the positive and negative symbols have been put to indicate whether the balance was in favour or against. The significant feature is that lime balance has been invariably negative in spite of feeding calcium carbonate. The magnesia balance has been positive and it does not call for any comment. The negative potash metabolism under rice straw and mustard cake has been generally a common feature and this is corroborated here. The ingestion of phosphorous has been nearly double the requirements and more than half of it has come from the share of Boro straw. The balances have been 50:50 positive. This also points to previous indications that the phosphorous of rice straw is of doubtful utility. *Soda and nitrogen* have been all positive under 'No lime' but under 'Lime' group one has recorded negative balance for soda and another for nitrogen. The addition of lime has not contributed favourably. It has been already noted that it has also depressed the availability of T. D. N. and starch equivalent. It has been found in many experiments here that the lime requirement under rice straw is always high. In a previous paper by Carbery, Chatterjee and Talapatra [1937] it was stated on the basis of experiments on Aus straw that the lime requirement per 500 lb. live weight is about 24 gm. CaO. The negative values in the cake of Boro straw even when the ingestion was over 29 g.m. suggest that the requirement under this straw is still higher. It is difficult to say what are the contributory causes. The potash content in this straw is high, but similar amount is found in the other straws also. It however contains a very large amount of oxalic acid as can be seen from the following table.

Table VI

Oxalic acid content of different straws

Laboratory No. of the straw	Straw	Oxalic acid crystal per cent	Lime equivalent to oxalic acid per cent.	Actual lime (CaO) per cent
61/1939	Amon straw, Dacca	0·662	0·294	0·425
62/1939	Aus straw, Dacca	0·646	0·287	0·643
47/1936	Boro straw, Dacca	1·496	0·665	0·343

The above results show that the oxalic acid present in Boro straw is considerably more than in Aus and Amon straws and further that it is theoretically capable of rendering insoluble nearly twice the amount of lime actually present. Recent work by Talapatra *et al* [1942] suggests that the soluble oxalic acid is probably largely decomposed in the reumen. Nevertheless the presence of a large amount of a substance which is both toxic and precipitant of lime is an unsatisfactory feature. The experiment suggests that the lime requirement per 500 lb. live weight under Boro straw is highest (not less than 30 gm. CaO), Aus comes next with 24 to 25 gm. CaO and Amon still less (about 18 to 20 gm.).

If we leave aside the unsatisfactory behaviour of lime metabolism as well as the high content of potash and oxalic acid, this straw like Aus variety is superior to Amon. Moreover it is the richest amongst all the rice straw varieties in protein content and in this respect it is even superior to Aus. It has been already pointed out that this straw contains a large percentage of insoluble siliceous matter. It means that a very large ingestion of such material is inseparable with its feeding. The trial which was conducted for a few weeks does not permit any inference as to how far a continuous feeding of this nature can have a cumulative effect.

It should also be stated here that the animals were fed sodium chloride at the rate of 25 gm. of sodium chloride per animal. There was no reason for a deficiency of chlorine. In spite of it there has been a negative balance, which apart from this trial has been noticed in the experiment with a few of other samples Amon rice straws obtained from the different soil belts (specially the saline tracts). At present no explanation can be offered for such a phenomenon.

SUMMARY

1. The feeding value of Boro variety of rice straw has been investigated and comparative data have been presented.

2. The experiment was conducted with 'No lime', and 'Lime'. The lime was given a calcium carbonate. The feeding of lime did not offer any advantage. On the other hand there was a depression of digestibility which was well marked in ether-extract and crude fibre, the former also exhibiting great fluctuation.

3. The main feature of this straw is a large percentage (compared to other rice straws) of protein content, a lower percentage of fibre, a very large percentage of insoluble silica and also a high percentage of oxalic acid.

4. Like Aus rice straw, Boro rice straw is definitely superior to Amon rice straw in its nutritive value on the organic side. As it contains the highest percentage of protein compared to the other two straws, it definitely economises protein requirement and from that standpoint is superior even to Aus straw.

5. Its unsatisfactory feature is that, its oxalic acid content is highest for which its lime requirement is highest (amongst all rice straws); and for reasons still unknown the chlorine balance has also exhibited a negative tendency besides lime, potash and phosphate which are usually so found in other rice straws.

6. Further experiments are required to devise correctives for the unsatisfactory lime, potash and chlorine metabolism as well as for high content of oxalic acid and also to see how the large quantity of insoluble siliceous matter as found in Boro straws, is likely to react on the animal system specially under a prolonged feeding.

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COMPOSITION OF FREE FATTY ACIDS IN HIGH ACID GHEE

By T. M. Paul, V. R. Bhalerao, C. P. Anantakrishnan and M. C. Rangaswamy, Imperial Dairy Research Institute, Bangalore

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CLARIFIED butterfat is commonly known as *ghee* in India and it is a very popular article of diet among the people. More than 50 per cent of all the milk produced in the country is utilized for the manufacture of *ghee*. Possibly due to faulty methods of manufacture, collection and storage conditions, very often *ghee* containing considerable amounts of free fatty acids, are come across in the market. These are often-times condemned as unfit for human consumption. During the course of an investigation into the causes of the development of acidity in *ghee*, it was thought interesting to know the nature of the free fatty acids present in *ghee*. Therefore, attempts were made to separate the free fatty acids from high acid *ghee* and to study their chemical composition.

In India butter is mostly prepared in small amounts and accumulated for various lengths of time before converting into *ghee*. It is possible that acidity is produced during this storage period. It was found that butter prepared from raw milk developed acidity in a shorter time compared to butter prepared from boiled milk. Therefore, butter was prepared from raw cow's milk by the indigenous method of souring the milk by addition of small amount of starter and then churning it. This butter was kept at room temperature for about a month. By that time the butter had developed about 20·0 per cent acidity calculated as oleic acid. The butter was then melted and converted into *ghee* by the 'boiling off' method. About 300 gm. of this *ghee* was set apart to study the chemical composition. This is referred to as Sample I. The rest of the *ghee* was boiled with twice its weight of 95·0 per cent alcohol, and the free fatty acids neutralized with dilute alkali. After separating the residual fat (Sample II), as far as possible, the soap solution was distilled to remove most of the alcohol. The alcoholic residue was diluted with water and extracted with successive portions of ether to ensure the removal of all fat from the soap. It was then acidified with hydrochloric acid and the liberated fatty acids were extraced exhaustively with ether.

The ether extracts were washed free from acids and the washings were collected (Solution K). The washed ether extracts were dehydrated, filtered and solvent (Solution Y) distilled off. The fatty acids (Sample III) were then converted into methyl esters by refluxing for one hour with 1,000 ml. of methyl alcohol containing 3 sulphuric acid. Most of the alcohol was then distilled off, the distillate made up to a known volume (Solution W) and preserved for subsequent analysis. The residue was cooled, transferred to a separating funnel and diluted with water. It was extracted exhaustively with ether. The ether extracts were washed free from acid. The washings were then mixed with solution K, made alkaline with KOH, and the volume was reduced at atmospheric pressure. The residue was acidified and steam distilled and the distillate (Solution X) titrated against 0·1 N alkali and expressed as methyl butyrate. The washed ether extracts were dehydrated filtered and solvent distilled off. (The distillate was mixed with solution Y and made to known volume). Aliquots were then taken from solution W and Y distillates and saponified with potash and the results expressed as methyl butyrate. Total of these determinations gave the amount of C_4 acid originally present in the fat. The methyl esters of the fatty acids now obtained and the methyl esters prepared from samples I and II were subjected to detailed ester fractionation in an EHP column according to the method of Hilditch as modified by Smith and Dastur [1938]. The results obtained are presented in Tables I to VI. The analytical characteristics of Sample I and II are as follows :

	Melting Point	B.R. Reading at 40°C.	Sap. Equivalent	Iodine Value	R.M.	Polenske	Free Fatty acids as oleic acid. (per-centage)
Sample I . .	33·2	40·2	246·8	29·55	25·85	1·65	19·31
Sample II . .	41·8	40·8	251·1	21·38	21·96	1·45	0·02

The data indicates that the refined sample possesses comparatively high melting point, possibly due to the presence of more of higher saturated glycerides. The iodine value in Sample II is very low indicating a reduction in the amount of unsaturated glycerides. The low R.M. value of refined sample can be attributed to the decrease in the steam volatile fatty acids. The acidity of the refined sample is negligible, confirming the complete removal of free fatty acids. From the Tables IV and V it is clear that the percentage of lower acids up to C_{14} for Sample I and II are 23·4 and 22·7 respectively. This shows that no appreciable change has taken place in the amount of lower acids except butyric acid which is reduced by 0·6 per cent due to refining. This is confirmed by the presence of higher amounts of butyric acid in the free acids as shown in Table VI. The amount of capric acid in both samples I and II is negligible. The sum of myristo-palmito-stearic group in Sample I is 53·0 per cent as against 62·8 in Sample II. Relatively all these acids are proportionately increased in Sample II. This is obvious as the amount of oleic acid is reduced by 9·9 per cent due to the removal of the free fatty acids from the original sample. Further, this accounts for the higher melting point of Sample II. The amount of arachidic acid in both the samples remains constant.

TABLE I

The fractionation of the methyl esters prepared from 250 gm. of high acid ghee

Fraction	B.P. at 2 m.m. up to	Percentage of total esters	Mol. wt.	I.V.	Fraction	B.P. at 2 m.m. up to	Percentage of total esters	Mol. wt.	I.V.
				Lower esters					
1	(Methyl butyrate)	4·29	..	..	7	137	1·12	219·9	10·02
2	85	0·59	152·3	3·11	8	143	1·44	229·5	10·49
3	98	1·01	165·7	6·10	9	147	1·46	236·0	10·87
4	105	1·28	180·7	8·04	10	150	1·66	242·9	10·47
5	114	1·46	189·9	11·77	11	153	1·29	249·2	10·44
6	124	1·47	199·9	11·51	12	155	1·98	257·8	10·64
						Total	19·05		
				Solid esters					
13		1·53	254·2	0·60	20	168	3·79	276·3	2·49
14	160	2·05	265·4	0·85	21	168	3·71	283·5	3·89
15	162	3·26	271·4	1·28	22	170	3·30	288·5	6·42
16	165	3·09	273·9	1·56	23	170	3·28	291·0	5·52
17	165	3·69	274·0	1·97	24	171	4·31	291·5	6·88
18	166	2·09	274·9	2·25	25	Residue	2·65	322·5	7·38
						Total	41·02		
19	168	3·37	274·9	2·31					
				Liquid esters					
26	148	1·44	233·9	19·89	35	172	2·03	286·5	71·04
27	155	1·56	248·8	22·24	36	273	2·36	286·9	71·12
28	165	2·83	261·1	38·42	37	175	2·20	289·0	72·70
29	168	1·90	279·1	55·70	38	177	2·18	290·2	74·19
30	168	1·93	281·6	59·75	39	176	3·22	291·4	76·60
31	168	2·23	283·2	68·73	40	177	3·16	293·0	77·85
32	170	1·86	285·6	69·05	41	178	3·23	296·2	79·77
33	171	2·00	285·8	69·10	42	Residue	3·59	324·5	76·07
34	172	2·27	285·	69·26		*Total*	39·93		

TABLE II

The fractionation of the methyl esters prepared from 250 gm. of Sample II

Fraction	B.P. at 2 m.m. up to	Percentage of total esters	Mol. Wt.	I.V.	Fraction	B.P. at 2 m.m. up to	Percentage of total esters	Mol. Wt.	I.V.
				Lower esters					
1	(Methyl butyrate)	3·72	..	..	6	132	1·17	210·8	9·59
2	78	0·88	156·6	2·72	7	144	1·43	226·2	8·77
3	88	1·07	173·0	5·53	8	151	1·14	238·6	8·10
4	96	0·82	187·1	9·16	9	155	1·05	247·2	8·81
5	115	1·10	196·0	10·60		*Total* .	12·29		
				Solid esters					
10	154	1·30	246·6	0·97	16	168	3·16	276·0	2·62
11	159	2·88	264·2	1·06	17	168	3·54	278·6	2·76
12	162	2·48	266·1	1·31	18	170	3·22	278·8	3·51
13	165	3·30	270·3	1·27	19	172	3·04	281·1	4·18
14	165	3·27	270·5	2·55	20	172	3·73	291·2	7·24
15	167	3·56	272·5	2·55	21	Residue	3·84	314·7	7·60
						Total .	37·32		
				Liquid esters					
22	144	1·95	231·5	9·48	31	175	2·99	287·4	44·30
23	153	1·68	246·5	9·15	32	177	3·40	288·4	52·27
24	159	2·59	254·9	8·89	33	178	3·33	289·4	52·60
25	162	2·81	261·0	22·20	34	178	3·17	290·8	52·56
26	165	3·62	265·5	27·19	35	178	3·33	291·0	61·00
27	166	3·05	268·2	28·76	36	179	2·78	292·9	61·30
28	168	2·98	269·0	28·73	37	180	2·73	296·1	61·95
29	170	3·18	273·8	43·91	38	Residue	2·71	308·9	61·06
30	172	4·20	274·8	43·94		*Total* .	50·20		

TABLE III

The fractionation of the methyl esters prepared from 100 g. of free fatty acids separated from high acid ghee

Fraction	B.P. at 2 m.m. up to	Percentage of total esters	Mol. wt.	I.V.	Fraction	B.P. at 2 m.m. up to	Percentage of total esters	Mol. wt.	I.V.
				Lower esters					
1	(Methyl butyrate)	4·91	..	..	5	133	1·17	222·7	21·95
2	85	1·29	166·3	19·63	6	140	2·32	243·3	10·32
3	102	1·24	187·4	15·92	7	147	2·78	246·5	18·28
4	118	1·37	208·0	17·70	8	153	3·80	258·6	24·39
						Total .	18·98		
				Solid esters					
9	160	2·11	226·2	0·71	12	175	4·55	275·1	3·15
10	168	2·72	266·2	0·74	13	Residue	9·15	303·3	15·37
11	172	3·17	271·7	1·43		*Total* .	21·70		
				Liquid esters					
14	152	1·50	258·1	33·20	20	175	7·24	292·8	82·02
15	160	2·88	271·0	48·79	21	176	6·70	295·5	85·41
16	165	3·66	284·6	69·12	22	178	6·19	295·6	85·80
17	168	4·23	288·6	69·72	23	178	4·00	295·7	85·90
18	172	5·15	289·4	77·14	24	177	5·66	295·3	89·60
19	174	6·33	290·7	80·13	25	Residue	5·78	309·8	83·22
						Total .	50·32		

TABLE IV

Summary of the calculated composition of the esters and fatty acids of high acid ghee

Acids	Per cent as methyl esters				Fatty acids (excluding unsaponifiables)	
	Lower	Solid	Liquid	Total	Per cent (Wt.)	Per cent Molar
Saturated.						
C_4	4·29	..	..	4·29	4·0	10·7
C_8	0·19	..	..	0·19	0·2	0·4
C_8	1·33	..	..	1·33	1·3	2·1
C_{10}	3·02	..	..	3·02	3·0	4·1
C_{12}	2·32	..	..	2·32	2·3	2·7
C_{14}	5·46	1·15	4·08	10·69	10·7	11·1
C_{16}	1·23	23·93	2·46	27·62	27·2	25·2
C_{18}	..	12·26	2·62	14·88	15·1	12·6
C_{20}	..	1·94	..	1·94	2·0	1·5
Total	17·84	39·28	9·16	66·28	65·8	70·4
Unsaturated.						
C_{10}	0·27	..	..	0·27	0·3	0·4
C_{12}	0·36	..	..	0·36	0·4	0·4
C_{14}	0·44	..	0·60	1·04	1·2	1·3
C_{16}	0·14	..	3·60	3·74	3·8	3·6
Oleic	..	1·74	25·02	26·76	26·9	22·6
Linoleic	..	..	0·17	0·17	0·2	0·2
C_{20}	..	..	1·38	1·38	1·4	1·1
Total	1·21	1·74	30·77	33·72	34·2	29·6
Sum of the saturated and unsaturated acids	19·05	41·2	39·93	100·00	100·0	100·00

TABLE V

Summary of the calculated composition of the esters and fatty acids of Sample II

Acids	Per cent as methyl esters				Fatty acids (excluding unsaponifiables)	
	Lower	Solid	Liquid	Total	Per cent (wt.)	Per cent (Molar)
Saturated.						
C_4	3·72	..	..	3·72	3·4	9·2
C_8	0·05	..	..	0·05	0·1	0·1
C_8	1·27	..	..	1·27	1·2	2·0
C_{10}	1·90	..	..	1·90	1·9	2·6
C_{12}	2·23	..	0·69	2·92	2·9	3·6
C_{14}	2·31	2·03	7·78	12·12	12·1	12·7
C_{16}	0·22	23·73	8·82	32·77	32·9	30·7
C_{18}	..	8·10	9·60	17·70	17·8	15·0
C_{20}	..	1·95	..	1·95	2·0	1·5
Total	11·70	35·81	26·89	74·40	74·3	77·3
Unsaturated.						
C_{10}	0·20	..	..	0·20	0·2	0·3
C_{12}	0·20	..	..	0·20	0·2	0·2
C_{14}	0·17	..	0·54	0·71	0·7	0·8
C_{16}	..	..	6·47	6·47	6·5	6·1
C_{18}	..	1·49	15·39	16·88	17·0	14·4
C_{20}	..	..	1·11	1·11	1·1	0·9
Total	0·57	1·49	23·51	25·57	25·7	22·7
Sum of the saturated and unsaturated acids	12·27	37·30	50·40	99·97	100·00	100·0

TABLE VI

Summary of the calculated composition of the esters of free fatty acids

Acids	Per cent as methyl esters				Fatty acids (excluding unsaponifiable)	
	Lower	Solid	Liquid	Total	Per cent (wt.)	Per cent (Molar)
Saturated.						
C_4	4·91	..	..	4·91	4·5	12·3
C_8	0·86	..	..	0·86	0·6	1·4
C_{10}	1·78	..	..	1·78	1·7	2·5
C_{12}	1·29	1·11	..	2·40	2·4	2·9
C_{14}	5·36	1·33	2·91	9·60	9·6	10·2
C_{16}	2·16	8·90	0·60	11·66	11·7	11·0
C_{18}	..	6·39	1·92	8·31	8·4	7·1
C_{20}	..	2·06	..	2·06	2·1	1·6
Total	16·36	19·79	5·43	41·58	41·2	49·0
Unsaturated.						
C_{10}	0·30	..	..	0·30	0·3	0·4
C_{12}	0·43	..	..	0·43	0·4	0·5
C_{14}	0·90	..	0·26	1·16	1·1	1·2
C_{16}	0·98	..	2·79	3·77	3·8	3·6
Oleic	..	1·91	48·07	49·98	50·4	43·2
Linoleic	..	..	0·28	0·28	0·3	0·2
C_{20-22}	..	..	2·49	2·49	2·5	1·9
Total	2·61	1·91	53·89	58·41	58·8	51·0
Sum of the saturated and unsaturated acids	18·97	21·70	59·32	99·99	100·0	100·00

The proportions of the lower unsaturated acids in high acid *ghee* appear to be of the same order as is found in normal butterfat, from cow's milk. It must be pointed out however, that while there is little doubt (from the trend of iodine value (of the lower ester fractions) that these minor unsaturated acid components exist, their quantitative percentage must be regarded only in the nature of a general indication of their true proportions, though not far from them. Sample II also gives the same proportion of these lower unsaturated acids.

Normally the free fatty acids are expressed in terms of oleic acid as this acid accounts for a large proportion of the free fatty acids. Davies [1941] from a preliminary study of the free fatty acids in high acid butterfat surmised that these are mostly composed of C_{16} and C_{18} acids, oleic acid appearing in the free form in the largest amount. The results reported herein show that the C_{18} and C_{16} acids account for 59·1 and 15·5 per cent respectively of the free fatty acids of high acid *ghee*, the balance being made up of other acids from C_4 to C_{20}. The sum of all the acids up to C_{14} is 20·8 per cent, which is a little lower than in the case of the original sample; while the C_{16} content is markedly lower. The sum of the myristopalmito-stearic group is only 29·7 per cent. The lower unsaturated acids are also of the same order as are found in cow *ghee*. The existence of other acids, besides oleic acid, in the free-fatty acids is confirmed by the Iodine Value (54·21) of Sample III. Comparing the component acids of all the samples tested, it can, therefore, be concluded that the

trend of the fatty acid composition of the unrefined sample is midway between the composition of the refined sample and that of the free fatty acids.

SUMMARY

1. A sample of *ghee* (clarified cow butterfat) containing 19·13 per cent free fatty acids, the natural *ghee* and the free fatty acids separated from the high acid *ghee* were subjected to detailed ester fractionation in an E.H.P. column.
2. The high acid *ghee* was found to be of the same composition as normal cow butterfat.
3. The neutral *ghee* contained the same amount of the low molecular weight acids and more of the myristto-palmito-stearic group than in the high acid *ghee*.

The free fatty acids contained the following percentages of the various acids, butyric=4·5, caprylic=0·8, caprie=1·7, laurie=2·4, myristic=9·6, palmitic=11·7, stearic=8·4, arachidic=2·1, oleic=50·4, and other unsaturated acids=8·4.

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STUDIES ON THE CURING OF POISONOUS *JAWAR* (*SORGHUM VULGARE*) PERS.

By N. D. KEHAR AND S. K. TALAPATRA, Animal Nutrition Section, Imperial Veterinary Research Institute, Izatnagar

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JAWAR is ordinarily a nutritious and palatable fodder, but under conditions of drought, wilting and frost it developes large quantities of cyanogenetic glucosides. Benson and Subba Rao [1906], Mann [1919] and Sharma [1935] have reported several deaths of cattle due to *jawar* poisoning. Since *jawar* is grown over large areas and as the average farmer cannot afford to discard his affected crops without serious setback to his already lean fodder resources, enquiries were received from several field investigators whether poisonous *jawar* could in any way be fed to livestock. This investigation was undertaken to find an answer to this query.

EXPERIMENTAL

Due to the partial failure of monsoon in 1944 prussic acid was detected in *jawar* plants of the Institute farm in October. This provided sufficient material for exploratory studies. Fresh samples were first tested with picrate paper according to the A.O.A.C. [1940] procedure. Having detected the presence of appreciable amounts of prussic acid qualitatively, a quantitative estimation was made by the acid titration method [A.O.A.C., 1940]. Samples of *jawar* containing prussic acid were then cured by different methods to evolve a technique by which prussic acid could be decomposed into innocuous constituents. Accordingly a bulk sample of *jawar* was brought to the laboratory immediately after cutting. The prussic acid content of the representative fresh sample was estimated and the rest of the material was treated as follows:

(*a*) Dried in shade at room temperature varying from 25°-30°C. and prussic acid estimated at the end of (i) 24 hours, (ii) 48 hours, (iii) 5 days and (iv) 15 days.

(*b*) Dried in the sun for eight hours and then kept in the shade at room temperature overnight.

(*c*) Dried in the field for a week and raked every third day.

(*d*) Ensiled in pits and prussic acid determined periodically.

RESULTS AND DISCUSSION

Each sample of *jawar* under different conditions of treatment was analysed in duplicate both qualitatively and quantitatively. The average values are shown in Table I.

TABLE I

Prussic acid content of jawar *under different treatments*

On dry basis

Particulars of samples	Parts of the Plant	Prussic acid content in mg. per 100 gm. of plant	Picrate test
Green plant immediately after cutting	Whole plant	14·9	Highly positive.
	Leaf	14·7	,, ,,
	Stem	16·0	,, ,,
Dried in shade for 24 hours at room temperature (30°C. approximately)	Whole plant	4·5	,, ,,
Dried in shade for 48 hours at room temperature (30°C. approximately)	,, ,,	3·8	,, ,,
Dried in shade for 5 days (temperature 27° to 30°C.)	,, ,,	3·4	,, ,,
Dried in shade for 15 days (temperature 25° to 30°C.)	,, ,,	1·1	Positive
Dried for 8 hours in the sun and kept overnight in shade	,, ,,	1·2	,,
Dried for 7 days in the sun under field conditions	,, ,,	*Nil*	Negative

Table I reveals several points of interest. It may be seen that the amount of prussic acid present in the stem was comparatively more than that of leaf. Similar results were obtained on examining 12 samples from the same affected area. Bagchi and Ganguly [1941] also found stems of 16 to 36 inches long plants richer in hydrocyanic acid than the leaves. Acharya [1933], however, stated that the leaves contained more hydrocyanic acid. On drying the plant, at room temperature about 70 per cent of the prussic acid was lost after 24 hours, and 75 per cent after 48 hours. When drying was continued for five days in shade at room temperature (27° to 30°C.) the loss in the prussic acid content was 77 per cent. The plant could not be made absolutely free of prussic acid even on drying in shade for 15 days. Acharya (*loc cit*) also found that drying in shade had little effect on the prussic acid content. Swanson [1921], however, stated that the shade dried sorghum contained only traces of prussic acid.

A remarkable loss of prussic acid was observed when the plant was dried in the sun even for short periods. Eight hours drying reduced the prussic acid by 90 per cent. Seven days drying under field conditions rendered the plant absolutely free from prussic acid. Leather [1906] found that sun-drying did not decrease the amount of cyanogenetic glucoside and Acharya (*loc cit*) stated that sun-drying reduced the amount of prussic acid appreciably. Chopra and Badhwar [1940] also assume that the well dried plants are not dangerous. This procedure of drying in the sun and leaving the plants out in the open at night was repeated thrice with the same results. The whole area under *jawar* crop was cured like this and fed to cattle without any untoward effects.

An alternative method of curing was also tried by ensiling the affected plants. The fresh plants were cut into two and ensiled in two small experimental pits of 4 ft. × 4 ft. × 4 ft. Locally grown hay consisting mostly of *Saccharum spontaneum* (*kans*) was used as cover. Samples from one pit were examined after every 10 days for prussic acid. It may be seen from Table II that the plants ensiled for about a month lost the entire prussic acid content. It is likely that the increased acidity and temperature in ensiling liberates the prussic acid from its combination with the enzyme and it is subsequently decomposed.

TABLE II

Hydrocyanic acid in ensiled samples of jawar

On dry basis

Particulars		mg. of HCN per 100 gm.
Pit No. I	Fresh sample	14·9
	10 days ensiling	5·2
	20 days ensiling	1·0
	30 days ensiling	*nil*
Pit No. II	Two months ensiling	*nil*

The ensiled sorghum from the second pit opened after two months was fed to three bullocks for a period of 30 days with no harmful effects. The animals relished the succulent fodder.

Although there is no experimental evidence, Van Der Walt [1944] has drawn attention to a condition of chronic prussic acid-poisoning if improperly cured sorghum is fed over a prolonged period. Similar views have been expressed in the *U.S.A. Veterinary Science News Letter* [1944] that small amounts of cyanogenetic forage is capable of causing demyelination if repeatedly consumed.

SUMMARY

Attempt has been made to utilize poisonous *jawar* as fodder by different treatments. It has been found that drying the affected plants in the sun for seven days or ensiling for 30 days renders the plant free from prussic acid.

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INVESTIGATIONS ON EVAPORATED BUFFALO MILK

By C. P. Anantakrishnan and Zal R. Kothavalla, Imperial Dairy Research Institute, Bangalore

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EVAPORATED milk is condensed milk to which no extraneous sugar has been added and which has been sterilized in hermatically sealed cans. Much attention has been paid towards the study of the preparation and properties of the evaporated cow's milk, but very little work has been done on the milk of other animals. In India, buffaloes form an important source of milk and dairy products and the milk and butterfat from these animals have formed the subject of many investigations. However, no information regarding the suitability of buffalo's milk for condensing seems to be available. Therefore, investigations were carried out to get information as to (1) the possible method of finding out the stability of evaporated milk during sterilization and (2) the applicability of 'rapid tests' which are at present in general use (for cow's milk) for predicting the heat stability of evaporated buffalo milk.

Experimental

The main factors contributing towards the success of milk condensing are uniform behaviour of liquid milk and uniform composition. Uniform composition is important in controlling the degree of condensing aimed at, which is generally done by determining the specific gravity. The standard in Great Britain for evaporated milk is 9 per cent fat and 22 per cent solids-not-fat. This gives a Fat : S.N.F. ratio of 1 : 2·44 which nearly conforms to the ratio for the average composition of fluid milk and allows for a slightly lower fat content. The United States standards are lower than the above. Previous to July 1940 the minimum standards for evaporated whole milk were 8 per cent fat and 28 per cent total milk solids. In the present investigation the fat : solids-not-fat ratio aimed at is 9 : 22. In order to obtain this ratio in the raw milk and to facilitate the control of the degree of concentration reached later, the liquid milk is toned to 4·0 per cent fat always, unless otherwise stated. The milk used in all cases was a composite sample from the Murrah buffalo herd of the Institute farm.

Milk was always taken for condensing four hours after production. Condensing was done in a pilot condensing unit manufactured by The Dairy Engineers Ltd., Edinburgh. Forewarming was effected by steam in a tinned copper kettle with revolving agitator, evaporation in a tinned copper vacuum pan, and condensation in a surface condenser. The vacuum is created by means of a wet vacuum pump.

Vacuum pan operation

The vacuum pan was thoroughly rinsed with water and steamed for 10 minutes. The air valves were closed, and water turned into the condenser. Then water was allowed into the vacuum pump and the pump was started. When the vacuum gauge registered over 62 cm. the valve of the milk pipe leading to the pan was closed. When the milk just filled the jacket and when it touched the steam coil in the pan, steam was turned into it. When all the milk was drawn into the pan the inlet was closed and the steam pressure in the jacket was increased gradually and taken up to 22 lb. in about 6 minutes. The temperature of the vacuum pan was maintained between 122 to 128°F. If the inside temperature of the pan was increased more than 128°F. initially, milk would begin to froth too much and might even be carried on to the condenser. Therefore, the steam pressure in the jacket should be carefully controlled. It has been found by various trials that a steam pressure of 22 lb. in the coils was quite suitable for condensing the milk. The temperature of the outlet water from the condenser ranged from 110 to 115°F. Just before the desired concentration was reached (judged by looking through the man-hole) a sample was drawn out from the pan and its specific gravity determined at a temperature of 110°F. When the specific gravity was about 1·066, the steam pressure in the jacket and the coils was reduced, the vacuum break opened, the vacuum pump and the steam in the jacket were stopped. The condensed milk is then drawn out of the pan and cooled to room temperature 72·76°F.

After standardization to the desired composition, the evaporated milk was filled in 8 oz. sterile vent hole cans, or in sterile glass bottles plugged with cotton wool and used for sterilization studies.

Forty lb. of standardized milk was used for condensing purposes and it generally took 20 to 25 minutes to complete one batch.

Sterilization

Sterilization was always carried out in an autoclave within two hours after condensation. The 'coming up' time, i.e. raising the temperature of the autoclave from room temperature to 240·8°F. or 248°F. was always adjusted between 20 to 25 minutes. The time taken from starting the heating of the autoclave to the point of filling steam was generally 10 to 14 minutes, and from this time the heating was carefully done to raise it to the required pressure and temperature, and in no case were the above limits relaxed. After holding the samples at various temperatures and for different lengths of time, cooling was effected in such a way that the samples came to room temperature in 20 minutes. In order to make the results as nearly comparable as possible it was necessary to choose an arbitrary rate of time at which to raise the temperature of the sterilizer to the temperature at which sterilization is to be effected.

Effect of concentration on the temperature of coagulation of evaporated buffalo milk

Forty lb. of milk toned to 4 per cent fat was taken for condensing and evaporated to various concentration. Not more than one sample of any two concentrations was prepared on the same day. Concentrations ranging from 18 to 22 per cent solids-not-fat were tried. For each concentration two samples on two different days were prepared. If the concentration exceeds the requirement, dilution was effected by distilled water. All milk samples were forewarmed to 203°F. for 5 minutes before concentration. Specific gravity, fat percentage and alcohol test of the raw milk were taken. The titrable acidity of the milk was expressed as percentage of lactic acid, the titration having been made with 0·1 N sodium hydroxide with phenolphthalein as indicator. The specific gravity, fat percentage, total solids and acidity of the concentrated milk were also taken. Sterilization of the evaporated milk was effected in 8 oz. vent hole cans. The coming-up period was 30 minutes in all cases. Duplicate samples of the two concentrations prepared on the same day were sterilized at temperatures 240·8, 244·4, 248·0, 251·6, and 257°F. for 30 minutes and the coagulation, if any, was observed. The results of these experiments are presented in Table I.

Table I

Effect of concentration on the temperature of coagulation of evaporated buffalo milk

Sample No.	Raw milk			Evaporated milk	
	S.N.F. per cent	Phosphate test	Alcohol test	S.N.F. per cent	Temp. of coagulation °F.
1	8·8	—	—	18·1	257·0
2	8·9	+	—	18·0	257·0
3	8·9	—	—	18·1	253·4
4	8·8	+	—	19·0	257·0
5	8·8	+	—	19·0	251·6
6	8·9	—	—	19·2	253·4
7	8·9	—	+	20·3	248·0
8	8·9	+	+	20·1	251·6
9	8·8	—	+	20·4	248·0
10	8·9	—	+	21·0	240·8
11	8·9	+	—	20·9	244·4
12	8·9	+	—	21·0	244·4
13	9·0	—	+	22·0	237·2
14	8·9	—	+	22·3	240·8
15	8·9	—	+	22·2	240·8

In general it can be concluded from Table I that as the concentration of the milk solid increases the temperature of coagulation for 30 minute sterilization decreases progressively, but there was no definite relationship between concentration and the change of coagulation temperature. A 22 per cent S.N.F. sample could be sterilized without coagulation between 237·2 to 244·4°F. for 30 minutes, but a few samples of 22 per cent S.N.F. which were not recorded in the table, coagulated even at 233·6°F.

Effect of various temperatures of sterilization on the time of coagulation of evaporated milk

Buffalo milk toned to 4 per cent fat was forewarmed at 203°F. for 5 minutes and condensed to 22 per cent S.N.F. The product was transferred to glass bottles and sterilized for various lengths of time at 233·6, 237·2, 240·8, 244·4, and 248·0°F. Four samples were tried at each temperature and the results are presented in Table II.

TABLE II

Time temperature relationship in the sterilization of evaporated buffalo milk

Sterilization temperature °F	Time of coagulation in minutes				
	Sample No.				
	1	2	3	4	Average
248·0	15	10	15	15	13·8
244·4	30	15	15	25	18·8
240·8	25	30	20	25	25·0
237·2	35	35	40	35	35·0
233·6	40	40	25	35	35·0

It can be seen from Table II that as the temperature of sterilization decreases the time required for coagulation increases for the product having 22 per cent S.N.F. The average time of coagulation for an evaporated milk of 22 per cent S.N.F. at 240·8°F. is about 25 minutes. The acidity of the samples of milk used in the above experiments ranged between 0·12 to 0·14 per cent. Six samples having 0·15 per cent acidity were found to coagulate at 233·6°F. in 25 minutes.

Effect of temperature of forewarming on the time of coagulation of the evaporated milk

The temperature and the time of forewarming of milk are known to have considerable effect on the heat coagulation of evaporated milk. Webb and Holm [1932] have shown that the concentration of S.N.F. is important in determining the effect of temperature and time of heating on heat coagulation. They conclude that forewarming at high temperatures lowers the stability of samples with high concentrations of S.N.F. It is also known that heating a milk to boiling prior to its evaporation strikingly increases the heat stability of its evaporated product. With a view to study the effect of forewarming on the stability of buffalo milk, various forwarming temperatures were tried. Buffalo milk toned to 4·0 per cent fat was forewarmed to 165, 185, and 203°F. respectively for five minutes and evaporated to 22·0 per cent S.N.F. and the time of coagulation at 240·8°F. was noted. Four samples were used at each forewarming temperature. The results of these experiments arel presented in Table III.

Table III shows that, as the temperature of forewarming increases, the time of coagulation at 240·8°F. increases. All the four samples which were not forewarmed at all curdled at 240·8°F. in less than five minutes. Forewarming definitely increases the heat stability of the evaporated buffalo milk and heating at 203°F. for five minutes is comparatively better than lower temperatures of forewarming. There is no marked physical thickening of the product prepared from milk forewarmed at 203°F. for five minutes, on storage for about three months. It was also of interest to

TABLE III

Effect of forewarming temperature on the heat stability of evaporated buffalo milk

Temperature of forewarming °F	Time of coagulation at 240·8°F. in minutes				
	Sample No.				
	1	2	3	4	Average
203	25	30	25	20	25·0
185	20	15	20	15	17·5
167	15	10	15	15	13·8
no forewarming	—	—	—	—	—

study the effect of forewarming at 203°F. for 10 or 15 minutes to find out whether the stability of the evaporated milk could be increased or not. Milk was forewarmed at 203°F. for 5, 10, and 15 minutes respectively and concentrated to 22·0 per cent S.N.F. The time of coagulation of these products at 240·8°F. was observed. The same sample of milk was used for forewarming at 5, 10 and 15 minutes on the same day. Three samples were tried in each case and the results are presented in Table IV.

TABLE IV

Effect of time of forewarming at 203°F. on the time of coagulation of evaporated buffalo milk

Time of forewarming in minutes	Time of coagulation at 240·8°F. in minutes			
	Sample No.			Average
	1	2	3	
5	25	20	25	23·8
10	20	20	25	22·5
15	15	20	15	16·2

Table IV shows that there is no significant difference in results between 5 and 10 minute period of forewarming at 203°F. but as the time of forewarning is increased to 15 minutes the time of coagulation decreases. The general conclusion drawn by foreign workers on cow's milk seems to show that as the time of forewarming is increased the heat stability is also increased (Rogers *et al*), but with buffalo milk this is not strictly so. This cannot be attributed to the acidity of the milk samples, for they ranged only between 0·12 to 0·14 as in other cases. Besides, in the case of milk which had been forewarmed at 203°F. for 15 minutes, the body of the finished product was thick in consistency and physical thickening was observed after a storage period of three months. But the evaporated milk prepared from milk forewarmed at 203°F. for five minutes was found to retain the creamy consistency even after keeping for one year. From the various experiments conducted so far, it has been found that the forewarming of milk at 203°F. for 5 minutes is the most suitable for the preparation of evaporated buffalo milk. The exact cause of the peculiar influence of forewarming temperature on the heat stability of buffalo milk cannot be explained at present.

Relation between phosphate and alcohol tests on the time of coagulation of evaporated milk

Forty lb. of buffalo milk toned to 4·0 per cent fat was forewarmed at 203°F. for five minutes, evaporated to 22·0 per cent S.N.F, and the time of coagulation at 240·8°F. was noted. Alcohol

and phosphate tests were carried out on milk samples before forewarming and condensing. Milk was considered alcohol positive when a precipitate appears with equal parts (2 ml.) of milk and 74 per cent alcohol. The ± point was taken as positive to 74 per cent alcohol and negative to 68 per cent alcohol. When negative to 68 per cent alcohol the milk was considered alcohol negative. The method of Ramsdell *et al* [1931] was adopted for the phosphate test. Ten ml. of milk sample were taken in a test tube of approximately 20 ml. capacity and one ml. of phosphate solution (68·1 gm. of mono basic potassium phosphate in one litre of water) was added to it. The contents of the tube were then mixed and the tubes immersed in a boiling water bath for 5 minutes. The tubes were then removed, cooled and the mixture examined for the presence of curd. Any visible coagulation indicated that the concentrated product would be of a low heat stability. Twenty-five trials were conducted with farm produced milk having acidity ranging from 0·12 and 0·15 per cent and the results are presented in Table V.

TABLE V

Relation of alcohol and phosphate test with the time of coagulation of evaporated buffalo milk

Number of trials	Alcohol test	Phosphate test	Coagulation time at 240·8°F. in minutes
8	—	—	23
7	—	+	20
4	+	+	16
3	±	—	22
3	±	—	14

From the above table it is seen that when the alcohol test is negative or ± the coagulation time ranges between 20 and 23 minutes. Eighteen samples out of 25 gave an indication from the alcohol test that they are fairly heat stable. Seven samples which gave positive alcohol tests coagulated within 16 minutes. Therefore, there is some relation between the alcohol test and heat stability of the evaporated product. Fourteen samples which gave negative phosphate tests coagulated at a temperature ranging from 14 to 23 minutes, while seven others which gave positive phosphate tests were fairly heat stable and four others which gave positive phosphate tests were not heat stable. Therefore, comparatively, the alcohol test appears to be more reliable in predicting the heat stability of the evaporated buffalo milk.

The effect of added salts on the heat coagulation of evaporated milk

The temperature at which evaporated milk curdles during sterilization is greatly affected and to a large extent controlled by the balance of milk salts. This fact is known in a general way but it remains to be investigated how added salts affect the heat stability of evaporated buffalo milk. Milk with 4·0 per cent fat was forewarmed at 203°F. for 5 minutes and condensed to 22·0 per cent S.N.F. In each of the 6 sterile bottles 140 gm. of the evaporated milk were taken, and one of the bottles was used as a control. To the others 0·2, 0·4, 0·6, 0·8 and 1·0 ml. of di-sodium phosphate solution (8·4 per cent strength) were added and well mixed. Wherever necessary the samples were adjusted for dilution by means of distilled water. All the samples were sterilized at 240·8°F. for 30 minutes. Similarly, the effect of sodium citrate of various concentrations on the heat coagulation of evaporated milk were also studied. Thus the effect of both these salts on the heat stability of evaporated milk was independently investigated. Twelve samples were tried and the results are presented in Table VI.

TABLE VI

Effect of stabilizers on the heat coagulation of evaporated buffalo milk at 240·8°F. for 30 minutes

Sample No.	·0	Amount of Na_2HPO_4 used in ml.				
		0·2	0·4	0·6	0·8	1·0
1	—	—	—	—	C	C
2	C	C	—	—	—	C
3	—	C	C	—	—	C
4	C	C	—	—	C	C
5	C	C	C	—	C	C
6	—	C	C	—	—	C
7	C	C	C	C	C	C
8	C	C	—	—	C	C
9	C	—	C	—	C	C
10	C	—	—	—	—	C
11	C	C	C	—	—	—
		Amount of sodium citrate in ml.				
1	C	C	—	—	—	C
2	C	C	—	—	—	C
3	C	C	C	C	—	C
4	C	C	C	—	—	C
5	C	—	—	—	—	—
6	C	C	—	—	—	C
7	—	—	C	—	—	C
8	—	—	—	—	—	C
9	—	—	—	C	C	C
10	—	C	C	—	C	C
11	—	—	—	C	—	C
12	C	C	C	C	—	—

C=curdled

Table VI reveals that evaporated milk to which 0·4 to 0·8 ml. of di-sodium phosphate were added, show good heat stability as compared with the lower or higher concentrations of the salt solution. The optimum concentration is found to be 0·6 ml. This corresponds to a concentration of 36 mgm. per 100 gm. of evaporated milk. But sodium citrate seems to require a different concentration. A concentration of 0·8 ml. is found to be the optimum. This suggests that for each batch of the evaporated milk a pilot sterilization is necessary to fix the amount of stabilizers to be used for safe sterilization.

Twelve samples of buffalo milk were obtained from a local farm and they were found to have acidities ranging from 0·14 to 0·18 per cent lactic acid. Eight samples gave a positive result to alcohol test. The samples were forewarmed at 203°F. for 5 minutes condensed to 22·0 per cent S.N.F. The sterilization at 240·8°F. for 20 minutes was effected with graded amounts of sodium bicarbonate in a way similar to that carried out with di-sodium hydrogen phosphate. It was found that though all the samples of evaporated milk were heat stable at a bicarbonate concentration of 0·8 ml. for every 140 gm. of evaporated milk, they all became brown in colour. For the other four samples sodium bicarbonate was found to be of no use and hence sodium citrate was tried. A concentration of 0·2 ml. for every 140 gm. of evaporated product was found to be suitable.

Evaporated milk sterilized at 240·8°F. for 25 minutes in vent-hole cans was eptk for one year and it was found to be in good condition.

Some of the evaporated milk samples prepared from the buffalo milk obtained from a local farm, though found to be heat stable were not completely sterile. They were examined and found

to contain spores and rod shaped organisms. Fresh samples of evaporated milk containing 22·0 per cent S.N.F. were inoculated with these spores and organisms before sterilization and heated to 248°F. for 20 minutes. After this process, the samples were incubated for 3 days at 98·6°F. to find out whether they survive or not. None of these samples were found to be sterile. In another trial the time of holding at 248°F. was increased to 30 minutes and then the samples incubated as before. It was found that these samples were practically sterile. But the holding time of 30 minutes at 248°F. for evaporated milk with a 22·0 per cent S.N.F. was found to be too much for this reason, that the final product curdled in the sterilizer. So, when these heat resistant spores get into milk, which is to be used for the manufacture of evaporated milk, the only way of getting at a wholesome sterile product is found to be, to concentrate the milk to only 19 per cent S.N.F. and sterilize at 248°F. for 30 minutes. But, this procedure would give a final product satisfying only the U. S. Standards for evaporated milk.

SUMMARY

The suitability of buffalo milk for the manufacture of evaporated milk was investigated.

It has been found, that as the concentration of the milk solids increases, the temperature of coagulation of the evaporated milk for 30 minutes sterilization decreases progressively.

For a product having 22·0 per cent solids-not-fat, as the temperature of sterilization decreases the time required for coagulation increases.

With milk containing 22·0 per cent S.N.F. the temperature of forewarming influences the time of coagulation. A five minutes forewarming at 203°F. was found to be the most suitable for buffalo milk.

The alcohol test was found to be more reliable than the phosphate test in predicting the heat stability of evaporated buffalo milk.

Stabilizers like di-sodium hydrogen phosphate and sodium citrate increase the heat stability of evaporated buffalo milk.

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NUTRITIVE VALUE OF RAW AND BOILED MILKS OF COWS AND BUFFALOES

By R. P. Singh, V. R. Bhalerao and C. P. Anantakrishnan, Imperial Dairy Research Institute, Bangalore

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(With one text-figure)

In India, different methods are adopted for processing milk. For house-hold consumption milk in some parts of India is brought to boil and allowed to simmer for about ten minutes before consumption. In certain other parts of the country, it is common to continue boiling for longer periods or even allow milk to simmer for over such long intervals as 10-12 hours. It is, therefore, of great importance from the point of view of human nutrition to determine whether any dimunition in the nutritive value of milk of either cow or buffalo is brought about by boiling. In foreign countries where a large proportion of milk is pasteurized the question as to whether or not heat treatment seriously diminishes the nutritive value of milk has been exhaustively studied. A large number of investigations are reported and the results are summarized by Stirling and Blackwood [1933] and Kon [1934]. It is generally concluded that pasteurization does not materially affect the nutritive value of milk. In holder pasteurization method, milk is not taken over 145° F. and the question arises whether taking milk to boiling temperature or simmering it as is usually done in our country, affects the nutritive value of milk. Henry *et al* [1938] studied the effect of commercial sterilization on the nutritive value of milk and found that there were no differences in the growth promoting values of milks. The rats on sterilized milk consumed it more readily than their mates on raw milk. Mitra [1942] studied the growth promoting and biological values of procins of raw cow and buffalo milk and concluded that there was no difference in their growth promoting values. In the present investigation, the common method of processing milk namely boiling for ten minutes has been adopted to determine the effect of heat treatment on the nutritive value of milks of cow and buffaloes.

Experimental

Raw milk was collected daily for the feeding experiments from the whole bulk of cow and buffalo milks from the herd of the Imperial Dairy Research Institute. A part of the sample was heated to boiling and simmered for about ten minutes, taking care not to allow any skin to be formed on the surface by keeping the liquid continuously stirred. It was then cooled taking the same precaution. The buffalo milk was toned with separated milk to the same fat percentage as cow milk before heating.

To study the nutritive value, the technique of Henry and Kon [1937] was adopted. Young rats were weaned when 21 days old and placed in experimental cages when 28 days old. Twelve male litter mates from 12 animals were used for each group and were fed exclusively on raw or boiled cow or buffalo milk, supplemented with minerals. Iron was supplied in the form of ferric chloride, and copper and manganese as sulphates. The minerals were administered per animal per day as follows :

First week—0·5 mg. Fe ; 0·05 mg. Cu.

Second week—0·5 mg. Fe ; 0·05 mg. Cu ; 0·04 mg. Mn.

Third week and subsequently—1·5 mg. Fe ; 0·15 mg. Cu ; 0·15 mg. Mn.

The animals were fed *ad libitum*. During the first two weeks the minerals were placed in small amount of milk fed in the morning and additional milk supplied in the evening. From third week 30 ml. of mineralized milk was supplied in the morning and the additional untreated milk in the evening depending on the amount consumed on the previous day. The rats were weighed every fourth day. The experiment lasted for two months, at the end of which period the animals were killed by anaesthetising and the body length measured from the tip of the nose to the centre of the anus.

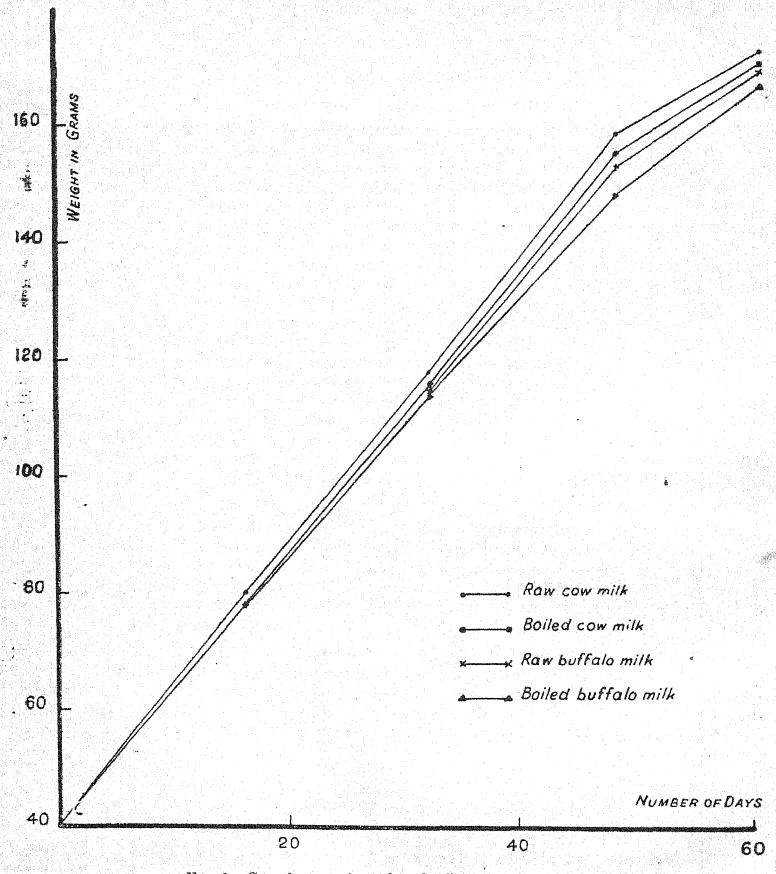

FIG. 1. Growth promoting value of milks (mate rats).

The results of the experiment are shown in Fig. 1, which shows the growth curve for animals reared on four types of milks. In Tables I, II, III and IV are shown the total gain in weight, gain in weight per day, milk consumed, gain in weight per 100 ml. of milk consumed over a period of four and eight weeks and the body lengths of the animals. The statistical data are shown in Table V.

TABLE 1

Result of the experiment of feeding rats on raw cow milk

Litter No.	Initial	Body weight of the rats in gm.		Total gain in body weight in gm.		Average gain in body weight per day in gm.		Milk intake in ml.		Gain in body wt. in gm. per 100 ml. of milk intake		Body length when killed (mm.)
		4 weeks	8 weeks	4 weeks	8 weeks	4 weeks	8 weeks	4 weeks	8 weeks	4 weeks	8 weeks	
1	40	122	182	82	142	2·56	2·37	1,362	2,992	6·02	4·86	192
2	40	126	167	86	147	2·56	2·45	1,370	3,038	5·98	4·84	200
3	40	118	173	78	133	1·94	2·22	1,328	2,988	5·87	4·45	193
4	40	117	170	77	130	2·41	2·17	1,351	2,944	5·70	4·42	195
5	40	120	178	80	138	2·50	2·30	1,369	3,007	5·83	4·59	191
6	40	112	163	72	123	2·25	2·05	1,321	2,144	5·53	4·33	192
7	39	113	165	74	126	2·31	2·10	1,367	3,037	6·31	4·15	194
8	37	115	163	78	126	1·94	2·10	1,321	2,935	5·90	4·29	190
9	37	111	172	74	135	2·31	2·25	1,343	3,003	5·51	4·50	191
10	37	116	166	79	129	3·11	2·15	1,343	2,945	5·88	4·38	190
11	40	116	176	76	136	2·38	2·27	1,362	3,026	5·58	4·49	197
12	40	114	160	74	120	2·31	2·00	1,350	2,808	5·48	4·27	195
Average	39·2	116·7	171·2	77·5	132·0	2·38	2·20	1,340	2,964	5·83	4·47	193·3

TABLE 2

Result of the experiment of feeding rats on boiled cow's milk

Litter No.	Initial	Body weight of the rats in gm.		Total gain in body weight in gm.		Average gain in body weight per day in gm.		Milk intake in ml.		Gain in body wt. in gm. per 100 ml. of milk intake		Body length when killed (mm.)
		4 weeks	8 weeks	4 weeks	8 weeks	4 weeks	8 weeks	4 weeks	8 weeks	4 weeks	8 weeks	
1	40	117	170	77	130	2·41	2·17	1,208	2,876	5·63	4·52	189
2	38	111	171	73	133	2·28	2·32	1,342	3,004	5·44	4·43	190
3	40	107	160	67	120	2·09	2·00	1,318	2,781	5·08	4·32	192
4	40	113	166	73	126	2·28	2·10	1,267	2,858	5·76	4·57	191
5	40	113	163	73	123	2·28	2·05	1,323	2,954	5·51	4·16	195
6	38	115	168	77	130	2·41	2·17	1,337	2,995	5·76	4·34	196
7	40	112	168	72	128	2·25	2·13	1,310	2,957	5·48	4·33	195
8	40	122	168	82	129	2·56	2·13	1,315	2,969	6·25	4·31	200
9	37	105	151	68	114	2·11	1·90	1,190	2,711	5·71	4·20	190
10	37	112	161	75	124	2·34	2·07	1,232	2,808	6·09	4·02	191
11	40	111	165	71	125	2·22	2·08	1,333	2,976	5·33	4·00	195
12	40	120	178	80	138	3·15	2·00	1,326	2,951	6·03	4·68	200
Average	39·2	113·2	165·3	74·0	126·1	2·36	2·11	1,299	2,903	5·70	4·37	193·7

TABLE 3

Result of the experiment of feeding rats on raw buffalo's milk

Litter No.	Initial	Body weight of the rats in gm.		Total gain in body weight in gm.		Average gain in body weight per day in gm.		Milk intake in ml.		Gain in body wt. in gm. per 100 ml. of milk intake		Body length when killed (mm.)
		4 weeks	8 weeks	4 weeks	8 weeks	4 weeks	8 weeks	4 weeks	8 weeks	4 weeks	8 weeks	
1	38	114	170	76	132	2·38	2·20	1,315	2,928	5·77	4·51	200
2	38	114	163	76	125	2·38	2·08	1,349	3,017	5·63	4·14	196
3	38	115	173	77	135	2·41	2·25	1,336	2,995	5·76	4·51	195
4	38	105	143	67	105	2·09	1·75	1,362	2,877	4·92	3·65	190
5	40	116	166	76	126	2·29	2·10	1,345	2,976	5·65	4·38	195
6	40	111	160	71	129	2·22	2·15	1,345	3,005	5·40	4·29	190
7	40	112	167	73	128	2·28	2·13	1,372	3,041	5·32	4·21	192
8	40	121	178	81	138	2·53	2·31	1,365	3,031	5·93	4·55	197
9	40	117	178	77	138	2·41	2·31	1,372	3,042	5·62	4·54	195
10	40	112	168	72	128	2·25	2·18	1,372	3,007	5·25	4·26	194
11	40	114	175	74	135	2·31	2·25	1,336	2,886	5·54	4·68	195
12	39	116	170	77	131	2·41	2·18	1,370	3,034	5·62	4·32	194
Average	39·2	113·9	168·2	74·7	129	2·34	2·16	1,353	2,987	5·53	4·34	194·4

TABLE 4

Result of the experiment of feeding rats on boiled buffalo's milk

Litter No.	Initial	Body weight of the rats in gm.		Total gain in body weight in gm.		Average gain in body weight per day in gm.		Milk intake in ml.		Gain in body wt. in gm. per 100 ml. of milk intake		Body length when killed (mm.)
		4 weeks	8 weeks	4 weeks	8 weeks	4 weeks	8 weeks	4 weeks	8 weeks	4 weeks	8 weeks	
1	40	113	173	73	133	2·28	22·2	1,350	2,971	5·38	4·48	186
2	40	121	189	81	149	2·53	2·48	1,348	3,004	6·01	4·96	192
3	39	118	176	79	137	2·47	2·20	1,354	2,995	5·83	4·58	191
4	39	115	171	76	132	2·38	2·28	1,367	3,014	5·56	4·38	190
5	39	120	167	87	128	2·53	2·15	1,323	2,891	6·12	4·43	189
6	39	119	168	80	129	2·50	2·15	1,372	2,971	5·83	4·34	195
7	40	119	176	79	136	2·47	2·27	1,372	3,038	5·76	4·47	194
8	40	111	168	71	128	2·22	2·13	1,354	3,001	5·25	4·27	191
9	39	107	153	68	114	2·11	1·90	1,302	2,860	5·22	3·98	185
10	39	112	163	73	124	2·28	2·07	1,273	2,830	5·73	4·91	191
11	38	110	165	72	127	2·25	2·42	1,270	2,877	5·67	4·41	185
12	38	109	167	71	129	22·2	2·15	1,221	2,742	5·82	4·70	185
Average	39·2	114·5	161·3	75·3	130·5	2·35	2·17	1,326	2,933	6·68	4·51	189·5

Table 5

Statistical treatment of the average gain in the weight at 4 and 8 weeks period and the body lengths of the experimental animals when fed exclusively on milk diet supplemented by minerals

	Gain in wt. in 4 weeks	Standard error of the mean	Gain in wt. in 8 weeks	Standard error of the mean	Body length when killed (mm.)	Standard error of the mean
Raw cow milk	77·5	±1·78	132·0	±2·69	193·3	±0·74
Boiled cow milk	74·9	±1·28	126·1	±1·62	193·7	±1·38
Raw buffalo milk	74·7	±1·31	129·0	±3·22	194·4	±1·17
Boiled buffalo milk	75·3	±1·48	130·5	±2·39	189·5	±0·43

	F Test for significance		
	In 4 weeks	In 8 weeks	Body lengths
Raw cow milk / Boiled cow milk	Not significant	Not significant	Not significant
Raw cow milk / Raw buffalo milk	Do.	Do.	Do.
Raw cow milk / Boiled buffalo milk	Do.	Do.	Significant
Boiled cow milk / Raw buffalo milk	Do.	Do.	Not significant
Boiled cow milk / Boiled buffalo milk	Do.	Do.	Significant
Raw buffalo milk / Boiled buffalo milk	Do.	Do.	Do.

Discussion

All the animals grew exceedingly well on the four types of milks. They were in good flesh, their coats were smooth and their eyes bright. It will be readily seen from Fig. 1 that rats fed on different milks show almost the same rate of growth. The animals receiving cow milk had grown a little better but the gain was not statistically significant. As seen from Tables I, II, III and IV, the differences in daily gains in weight made by rats fed on different milks are negligible. The efficiency of utilization of milk as measured by the gain in weight per 100 ml. of milk was also same for all the groups. The figures for milk consumption further revealed that there was no difference in the appetite of the rats in the various groups. Thus it is seen from the results of the experiment that boiling either cow or buffalo milk for about ten minutes had no appreciable effect on their growth promoting values. When the body lengths of the rats fed on these milks are taken into consideration after killing it was found that the mean body length of the rats on boiled buffalow milk was slightly less (by about 4·5 mm.) than those on either raw buffalo milk, raw cow milk or boiled cow milk. This slight difference, however, does not appear to be of any special significance.

SUMMARY

The male rats were fed *ad libitum* for a period of eight weeks on raw and boiled milks of cows and buffaloes supplemented with minerals. No differences were observed in the growth promoting values under the conditions of boiling, *viz.*, allowing the milk to simmer for ten minutes.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to Dr K. C. Sen, Director of Dairy Research, Mr M. C. Rangaswamy, Dairy Husbandry Officer and Dr Noshir N. Dastur, Dairy Chemist for their interest and useful suggestions in planning out this work.

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DETECTION OF ADULTERATION OF MILK WITH MILK POWDER

By K. N. Shama Sastry and Noshir N. Dastur, Imperial Dairy Research Institute, Bangalore

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In normal times cheap skim-milk powder is available and this is used freely in certain urban centre to tone whole milk rich in fat. If the milk powder solution is prepared carefully and tonning so carried out that the final product conforms to the legal standard for fat percentage in milk, this tonning cannot be detected by the routine methods normally used for testing milk. Nutritionally such tonning is not objectionable but there is no doubt that the product cannot be regarded as genuine milk. Further, such a practice has graver effects on the genuine liquid milk market than are apparent at first sight and requires to be curbed by all available means.

The problem of adulteration of milk with milk powder is peculiar to the urban centres in India. Studies have been, therefore, undertaken to find simple methods which will prove useful under household conditions and the laboratory. The results of these studies are given below.

Experimental

1. *Milk powders used*

Three brands of milk powders were used, *viz.*, spray-dried skim-milk powder (New Zealand); roller-dried skim milk powder (Benares); and roller-dried whole milk powder (Benares). Solutions of different powders were prepared as follows:

(i) *Spray-dried skim-milk powder.* Skim-milk powder, 9·5 gm., was sprinkled over 90 ml. of water (40°C.), shaken for a minute and filtered through muslin. The fat percentage of this solution was 0·1 per cent and the solids-not-fat content 8·4-8·5 per cent.

(ii) *Roller-dried skim-milk powder.* A quantity, 9·0 gm., of the powder were sprinkled over 90 ml. of water (60°C.), shaken for one minute, cooled and filtered through muslin. The fat percentage of the reconstituted skim-milk was 0·25 per cent and the S. N. F. varied from 8·4-8·5 per cent.

(iii) *Roller-dried whole milk powder.* Whole milk powder, 10·2 gm., was sprinkled over 90 ml. of water (60°C), shaken for a minute, cooled and filtered. The fat percentage of this solution varied from 1·4-1·6 per cent and the S. N. F. from 8·4-8·5 per cent.

In all cases it was aimed to get S. N. F. of about 8·5 per cent and so the quantity of powder to be used was varied accordingly.

2. *Preparation of tonned milk*

For preparing tonned milk, the fat percentage of fresh whole buffalo milk was brought to 4·0 per cent and that of fresh whole cow milk to 3·5 per cent by adding appropriate amount of respective milk powder solutions.

3. *Tests tried*

(i) *Stability to heat.* Pure samples of milk, both raw and boiled can easily be distinguished from similar samples of milk powder by their odour. However, such distinction could not be made so easily with tonned samples. Hence trials were carried out by incubating the samples for different lengths of time at 37°C. This temperature was selected as facilities are usually available for maintaining it in all public health laboratories.

In a series of experiments samples of milk and tonned milk, 50 ml. in glass stoppered bottles, were kept at 37°C. The flavour was compared at intervals of an hour over a period of eight hours. It was found that under these conditions tonned milk samples developed a peculiar burnt flavour which characterised them from other samples. This flavour became very pronounced after four hours incubation and longer intervals did not seem to make much difference in the intensity of flavour. This observation was made both with cow and buffalo tonned milks.

In the above experiments it was found that to distinguish tonned milk from pure milk, it was necessary to have samples of raw milk for comparison. Due to the development of acidity during incubation the burnt flavour in tonned milk became slightly masked. To overcome this, tests were

TABLE I

Results of rennet test with raw, boiled and tonned buffalo milk and raw and boiled spray dried separated milk
(Coagulation time in seconds)

Serial No.	Fat Percentage of original raw milk	Raw Samples												Bottled Samples											
		Original milk				Tonned milk				Milk powder milk used for tonning				Original milk				Tonned milk				Milk powder milk used for tonning			
		0	15	30	50	0	15	30	50	0	15	30	50	0	15	30	50	0	15	30	50	0	15	30	50
1	7·00	49	51	53	..	77	79	89	..	650	845	1177	..	136	156	189	..	..	..	..	..	1012	2340	1800	..
2	6·90	70	74	71	..	109	114	119	..	565	607	835	..	152	196	221	..	271	403	677	..	772	1339	1795	..
3	7·00	64	60	61	..	53	124	129	..	..	..	..	..	130	159	192	..	220	280	520	..	..	..	..	..
4	6·90	48	42	43	..	73	72	68	..	295	337	495	..	79	84	89	..	141	137	157	..	605	697	885	..
5	8·30	52	46	42	..	85	81	94	..	337	430	562	..	134	141	163	..	210	237	390	..	670	705	870	..
6	7·40	57	56	53	..	82	80	89	..	344	447	587	..	162	172	215	..	249	287	427	..	665	755	862	..
7	7·10	49	44	42	..	80	80	67	..	337	475	597	..	120	119	137	..	214	224	322	..	627	697	872	..
8	7·20	54	57	52	..	93	97	99	..	..	..	..	..	134	175	167	..	198	219	354	..	..	..	..	..
9	6·60	59	54	55	..	86	88	87	..	307	442	500	..	154	181	213	..	200	227	342	..	650	727	822	..
10	7·20	65	60	62	..	108	114	123	..	325	455	617	..	149	164	212	..	203	239	297	..	625	652	832	..
11	7·05	78	79	77	..	109	114	126	..	285	467	702	..	223	228	326	..	166	183	219	..	662	692	720	..
12	7·40	70	66	66	..	120	123	134	..	432	660	1050	..	171	179	201	..	224	268	341	..	697	1050	1095	..
13	6·70	49	48	46	..	69	67	73	..	390	660	990	..	156	176	212	..	110	112	134	..	570	1020	1290	..
14	7·50	37	36	30	..	63	58	56	..	517	720	1110	..	107	116	125	..	189	226	277	..	675	810	1350	..
15	6·90	50	49	50	..	85	82	91	..	495	750	1050	..	163	177	256	..	157	185	237	..	570	750	930	..
16	7·70	47	47	46	..	83	88	89	..	455	495	570	..	144	178	223	..	219	301	473	..	465	525	870	..
17	6·60	57	54	52	..	71	75	79	..	360	480	660	..	197	217	218	..	240	281	413	..	420	660	750	..
18	7·35	42	39	36	..	55	55	58	..	420	510	660	..	125	126	147	..	222	206	492	..	420	660	1080	..
19	7·85	50	47	46	..	95	91	106	..	..	..	..	..	123	126	154	..	206	287	515	..	..	..	..	..
20	7·50	43	39	37	..	88	91	106	..	345	417	435	..	127	123	156	..	214	204	502	..	405	465	510	..
21	7·80	35	31	31	..	56	56	57	..	345	450	630	..	141	154	198	..	183	239	362	..	420	510	690	..
22	7·60	41	42	42	..	92	90	99	..	315	375	555	..	141	149	183	..	202	267	397	..	435	510	780	..
23	9·60	41	40	40	..	121	133	154	..	345	435	517	..	132	139	159	..	223	297	622	..	465	510	570	..
24	6·10	62	60	59	..	88	95	105	..	..	..	..	..	161	199	242	..	206	287	465	..	..	..	..	..
25	6·70	54	54	57	..	97	99	114	..	345	390	435	..	137	155	151	..	226	284	418	..	450	555	840	..
26	7·05	53	50	49	..	93	92	96	..	360	368	480	..	138	146	180	..	251	330	422	..	681	969	1410	..
27	7·30	58	54	54	..	96	112	124	..	300	309	307	..	161	184	267	..	185	243	400	..	655	1020	1320	..
28	6·80	56	54	52	..	94	95	99	..	341	410	440	..	..	..	..	..	..	..	..	..	..	..	..	..
29	7·75	52	50	48	..	94	98	112	..	285	330	345	..	143	167	203	..	229	327	495	..	405	427	540	..
30	5·30	69	..	70	..	90	..	92	..	..	..	..	..	257	..	453	..	323	..	591	..	..	..	..	..

31	7·80	46	..	44	42	96	..	104	165																		
32	7·30	52	..	50	53	103	..	129	149																		
33	7·60	41	..	40	38	78	..	79	115																		
34	7·80	57	..	54	54	105	..	131	228																		
35	7·90	46	..	42	40	71	..	76	108																		
36	8·30	51	..	45	46	94	..	106	137																		
37	7·40	48	..	43	43	73	..	84	104																		
38	7·25	47	..	44	44	85	..	88	144																		
39	7·65	51	..	45	47	80	...	87	124																		
40	8·50	51	..	50	52	83	..	105	168																		
41	7·60	56	..	51	56	91	..	108	163																		
42	5·65	98	..	116	193	129	..	101	297																		
43	7·75	53	..	49	52	87	..	103	140																		
44	6·70	75	..	79	92	107	..	134	242																		
45	7·70	51	..	47	49	81	..	89	124																		
46	7·40	46	..	43	43	77	..	84	114																		
47	6·40	57	..	63	74	86	..	111	159																		
48	6·85	50	..	49	53	84	..	94	125																		
49	6·60	43	..	40	41	67	..	70	85																		
50	7·80	48	..	45	46	88	..	96	143																		
51	7·30	37	..	31	31	67	..	74	110																		
52	6·30	55	..	52	53	81	..	81	104																		
53	7·40	43	..	37	37	66	..	71	84																		
54	5·80	57	..	54	56	80	..	87	101																		
55	6·20	40	..	36	37	74	..	73	88																		
56	9·20	56	..	50	53	84	..	81	97																		
57	6·80	56	..	51	54	92	..	101	146																		
58	6·60	55	..	53	56	88	..	94	124																		
59	5·80	71	..	71	83	99	..	110	145																		
60	6·80	54	..	52	55	88	..	98	130																		
61	6·80	49	..	47	48	83	..	89	113																		
62	6·60	48	..	47	49	80	..	84	106																		
Average .		53	51	51	56	85	91	96	136	379	492	656	..	148	160	202	..	210	254	402	..	584	793	978	..		

TABLE I

Results of rennet test with raw, boiled and tonned buffalo milk and raw and boiled spray dried separated milk
(Coagulation time in seconds)

Serial No.	Fat Percentage of original raw milk	Raw Samples												Bottled Samples											
		Original milk				Tonned milk				Milk powder milk used for tonning				Original milk				Tonned milk				Milk powder milk used for tonning			
		0	15	30	50	0	15	30	50	0	15	30	50	0	15	30	50	0	15	30	50	0	15	30	50
1	7·00	49	51	53	..	77	79	89	..	650	845	1177	..	136	156	189	..	..	..	..	..	1012	2340	1800	..
2	6·90	70	74	71	..	109	114	119	..	565	607	835	..	152	196	221	..	271	403	677	..	772	1330	1795	..
3	7·00	64	60	61	..	53	124	129	..	..	..	..	..	130	150	192	..	220	280	530	..	..	..	..	..
4	6·90	48	42	43	..	73	72	68	..	295	337	495	..	79	84	80	..	141	137	157	..	605	697	885	..
5	8·30	52	46	42	..	85	81	94	..	337	430	562	..	134	141	163	..	210	237	390	..	670	705	870	..
6	7·40	57	56	53	..	82	80	89	..	344	447	587	..	162	172	215	..	249	287	427	..	665	755	862	..
7	7·10	49	44	42	..	80	80	67	..	337	475	597	..	120	119	137	..	214	224	322	..	627	697	872	..
8	7·20	54	57	52	..	93	97	99	..	..	..	..	..	134	175	167	..	198	219	354	..	..	..	..	..
9	6·60	59	54	55	..	86	88	87	..	307	442	500	..	154	181	213	..	200	227	342	..	650	727	822	..
10	7·20	65	60	62	..	108	114	123	..	325	455	617	..	149	164	212	..	203	239	297	..	625	652	832	..
11	7·05	78	79	77	..	109	114	126	..	285	467	792	..	223	228	326	..	166	183	219	..	662	692	720	..
12	7·40	70	66	66	..	120	123	134	..	432	690	1050	..	171	179	201	..	224	268	341	..	697	1050	1095	..
13	6·70	49	48	46	..	69	67	73	..	390	660	990	..	156	176	212	..	110	112	134	..	570	1020	1290	..
14	7·50	37	36	30	..	63	58	56	..	517	720	1110	..	107	116	125	..	189	226	277	..	675	810	1350	..
15	6·90	50	49	50	..	85	82	91	..	495	750	1050	..	163	177	256	..	157	185	237	..	570	750	930	..
16	7·70	47	47	46	..	83	88	80	..	455	495	570	..	144	178	223	..	219	301	473	..	465	525	870	..
17	6·60	57	54	52	..	71	75	79	..	360	480	660	..	197	217	218	..	240	281	413	..	420	660	750	..
18	7·35	42	39	36	..	55	55	58	..	420	510	660	..	125	126	147	..	222	296	492	..	420	660	1080	..
19	7·85	50	47	46	..	95	91	106	..	..	..	..	..	123	126	154	..	206	287	515	..	..	..	..	..
20	7·50	43	39	37	..	88	91	106	..	345	417	435	..	127	123	156	..	214	264	502	..	405	465	510	..
21	7·30	35	31	31	..	56	56	57	..	345	450	630	..	141	154	198	..	183	239	362	..	420	510	690	..
22	7·60	41	42	42	..	92	90	99	..	315	375	555	..	141	149	183	..	202	267	397	..	435	510	780	..
23	9·60	41	40	40	..	121	133	154	..	345	435	517	..	132	139	159	..	223	297	622	..	465	510	570	..
24	6·10	62	60	59	..	88	95	105	..	..	..	..	..	161	199	242	..	206	287	465	..	..	..	..	..
25	6·70	54	54	51	..	91	99	114	..	345	390	435	..	137	155	151	..	226	284	418	..	450	555	840	..
26	7·05	53	50	49	..	93	92	96	..	360	368	480	..	138	146	180	..	251	330	422	..	681	969	1410	..
27	7·30	58	54	54	..	96	112	124	..	360	309	307	..	161	184	267	..	185	243	400	..	655	1020	1320	..
28	6·80	56	54	52	..	94	95	99	..	341	410	440	..	..	..	..	..	..	..	..	..	..	..	..	..
29	7·75	52	50	48	..	94	98	112	..	285	330	345	..	143	167	203	..	220	327	495	..	405	427	510	..
30	5·30	69	..	70	..	90	..	92	..	..	..	..	..	257	..	453	..	323	..	591	..	..	..	..	..

31	7·80	46	..	44	42	96	..	104	165																
32	7·30	52	..	50	53	103	..	129	149																
33	7·60	41	..	40	38	78	..	79	115																
34	7·80	57	..	54	54	105	..	131	228																
35	7·90	46	..	42	40	71	..	76	108																
36	8·30	51	..	45	46	94	..	106	137																
37	7·40	48	..	43	43	73	..	84	104																
38	7·25	47	..	44	44	85	..	88	144																
39	7·65	51	..	45	47	80	..	87	124																
40	8·50	51	..	50	52	83	..	105	168																
41	7·60	56	..	51	56	91	..	108	163																
42	5·65	98	..	116	193	129	..	191	297																
43	7·75	53	..	49	52	87	..	103	140																
44	6·70	75	..	79	92	107	..	134	242																
45	7·70	51	..	47	49	81	..	89	124																
46	7·40	46	..	43	43	77	..	84	114																
47	6·40	57	..	63	74	86	..	111	159																
48	6·85	50	..	49	53	84	..	94	125																
49	6·60	43	..	40	41	67	..	70	85																
50	7·80	48	..	45	46	88	..	96	143																
51	7·30	37	..	31	31	67	..	74	110																
52	6·30	55	..	52	53	81	..	81	104																
53	7·40	43	..	37	37	66	..	71	84																
54	5·80	57	..	54	56	80	..	87	101																
55	6·20	40	..	36	37	74	..	73	88																
56	6·20	56	..	50	53	84	..	81	97																
57	6·80	56	..	51	54	92	..	101	146																
58	6·60	55	..	53	56	88	..	94	124																
59	5·80	71	..	71	83	99	..	110	145																
60	6·80	54	..	52	55	88	..	98	130																
61	6·80	49	..	47	48	83	..	89	113																
62	6·60	48	..	47	49	80	..	84	106																
Aver	*age* .	53	51	51	56	85	91	96	136	379	492	656	..	148	160	202	..	210	254	402	..	584	793	978	..

TABLE II

Results of rennet test with raw, boiled and tonned buffalo milk and raw and boiled spray-dried separated milk
(Coagulation time in seconds)

Serial Number	Fat Percentage of original raw milk	Raw Samples								Boiled Samples							
		Original milk				Tonned milk				Original milk				Tonned milk			
		Percentage Adulteration								Percentage adulteration							
		0	15	30	50	0	15	30	50	0	51	30	50	0	15	30	50
1	4·30	71	71	68	..	75	68	74	..	262	443	1290	..	137	172	244	..
2	4·40	52	49	51	..	53	46	46	..	261	391	1090	..	213	276	454	..
3	3·90	70	74	76	..	78	77	82	..	191	249	537	..	216	273	380	..
4	4·35	75	76	82	..	100	100	108	..	261	307	576	..	238	295	555	..
5	4·60	130	142	168	..	151	167	207	..	304	720	900	..	597	1170	1395	..
6	4·60	89	91	109	..	119	124	148	..	218	297	527	..	326	452	1012	..
7	4·60	112	121	141	..	139	147	201	..	297	487	1582	..	415	1010	1440	..
8	4·40	78	78	79	..	55	57	63	..	145	165	194	..	226	262	515	..
9	4·40	105	108	120	..	129	138	181	..	292	483	1300	..	347	597	1680	..
10	4·25	57	58	65	..	71	73	81	..	249	313	757	..	202	227	685	..
11	4·05	87	91	102	..	115	127	196	..	276	403	1177	..	357	686	1410	..
12	4·85	84	79	88	..	95	101	123	..	289	672	1800	..	425	930	1500	..
13	4·60	59	61	65	..	75	80	91	..	206	482	1027	..	372	527	862	..
14	5·00	114	313	115	..	144	161	264	..	362	537	870	..	432	875	1057	..
15	5·00	107	129	150	..	139	155	219	..	347	650	1110	..	362	485	810	..
16	4·20	41	45	43	..	45	45	48	..	226	352	875	..	253	452	1207	..
17	4·10	43	51	49	..	63	66	80	..	321	465	1167	..	400	1015	1590	..
18	4·50	94	101	124	..	129	138	195	..	417	1027	1530	..	505	810	950	..
19	4·90	48	52	50	..	71	72	87	..	149	214	317	..	212	387	887	..
20	3·60	104	114	134	..	122	137	184	..	393	787	1192	..	436	845	1477	..
21	4·90	113	124	151	..	180	223	357	..	522	875	910	..	667	790	1050	..
22	5·00	95	92	102	..	133	139	170	..	177	290	336	..	367	892	1380	..
23	4·45	110	120	133	..	141	160	255	..	417	545	1203	..	560	1350	1920	..
24	4·90	73	76	79	..	117	127	150	..	..	..	..	..	..	..	..	..
25	5·50	77	86	96	..	121	143	211	..	251	425	1080	..	532	732	1500	..

26	5·00	90	..	101	163	141	..	212	425								
27	6·85	50	..	49	50	94	..	122	203								
28	5·00	99	..	114	135	126	..	175	142								
29	5·75	79	..	92	126	131	..	187	327								
30	4·40	80	..	95	137	96	..	118	202								
31	4·70	95	..	117	196	125	..	169	317								
32	4·40	50	..	51	62	52	..	59	72								
33	4·65	77	..	97	152	106	..	166	317								
34	5·05	90	..	116	178	120	..	184	367								
35	5·15	104	..	130	131	136	..	202	412								
36	5·20	91	..	117	211	126	..	197	402								
37	5·05	100	..	114	156	120	..	171	332								
38	4·55	55	..	61	75	66	..	72	107								
39	5·05	81	..	104	147	113	..	165	242								
40	5·30	76	..	91	132	91	..	135	220								
41	4·55	93	..	123	231	121	..	193	377								
42	6·40	49	..	53	57	78	..	77	152								
43	4·90	104	..	129	238	139	..	195	377								
44	5·10	118	..	149	266	160	..	225	330								
45	5·20	75	..	78	101	101	..	120	186								
46	5·60	86	..	96	138	86	..	116	151								
47	5·20	81	..	97	150	125	..	171	287								
48	5·80	49	..	51	55	81	..	88	119								
49	5·30	91	..	108	154	131	..	166	272								
50	5·50	86	..	92	117	131	..	173	298								
51	5·30	67	..	130	170	124	..	209	279								
52	5·60	104	..	130	228	159	..	239	332								
53	5·50	87	..	95	78	153	..	233	347								
54	4·60	87	..	106	151	116	..	143	263								
55	5·30	115	..	158	253	176	..	232	407								
56	5·20	121	..	152	311	156	..	293	387								
57	5·15	99	..	118	190	150	..	238	313								
Average		86	89	101	154	116	115	168	284	288	477	950	..	366	646	1123	..

carried out as described above but before incubating a drop of formalin was added. By this modification of the test, the distinction between tonned and pure samples became very sharp and tonned milk could be detected without the slightest ambiguity.

In practice this test has proved to be the simplest and the most sensative test of those tried for distinguishing milk tonned with milk powder.

(ii) *Rennet test.* For this test 50 ml. of milk were brought to 37°C. and 1 ml. of 10 per cent Hansen's liquid rennet added, while stirring. The time when flakes first appear was noted.

Preliminary studies showed that diluting the milk under test with distilled water helped to differentiate between pure and tonned milk. Hence this method was adopted for all the studies described below. The results in detail are given in Table I to VI. The figures 0, 15, 30, etc., in the heading of the Table represent the percentage of distilled water added to milk (V/V).

TABLE III

Rennet test with buffalo milk and tonned buffalo milk (roller dried whole milk powder)
(Coagulation time in seconds)

Serial Number	Fat	S. N. F.	Raw samples					
			Original milk			Tonned milk		
	of original raw milk		Percentage adulteration					
			0	30	50	0	30	50
1	7·50	8·45	64	59	62	768	917	1125
2	5·25	8·50	85	98	112	217	310	375
3	6·90	8·50	76	84	97	412	952	1800
4	6·40	8·48	42	40	39	193	261	330
5	5·95	8·42	49	43	25	252	251	378
6	5·70	8·38	56	52	54	182	215	362
7	6·90	8·36	43	34	39	102	107	128
8	6·50	8·47	47	42	41	207	275	421
9	6·15	8·37	71	68	75	357	663	1800
10	5·50	8·35	67	71	80	199	264	435
11	5·90	8·42	66	62	58	314	417	777
12	6·50	8·36	60	57	67	323	566	1409
Average		8·42	60	59	62	294	433	778

TABLE IV

Rennet test with buffalo milk and tonned buffalo milk (roller dried skim-milk powder)
(Coagulation time in seconds)

Serial Number	Fat	S. N. F.	Raw samples					
			Original milk			Tonned milk		
	of original raw milk		Percentage adulteration					
			0	30	50	0	30	50
1	6·40	8·58	42	40	39	354	563	1120
2	5·75	8·53	78	72	83	597	947	1800
3	6·70	8·56	50	48	48	670	1082	1800
4	5·90	8·45	93	103	144	440	944	1800
5	5·95	8·52	49	43	25	444	725	911
6	5·70	8·56	56	52	54	351	477	722
7	6·90	8·17	43	34	39	302	405	831
8	6·15	8·60	71	68	76	563	1008	1800
9	5·50	8·61	67	71	80	270	402	795
10	6·50	8·42	60	57	67	554	1035	1800
11	5·70	8·50	56	54	59	383	564	605
12	7·30	8·41	45	44	48	263	333	508
Average		8·49	59	57	63	432	624	786

TABLE V

Rennet test with cow milk and tonned cow milk (roller dried whole milk powder)
(Coagulation time in seconds)

Serial Number	Fat	S. N. F.	Raw samples					
			Original milk			Tonned milk		
	of original raw milk		Percentage adulteration					
			0	30	50	0	30	50
1	5·70	8·60	112	138	222	275	375	432
2	4·80	8·42	83	99	128	322	382	445
3	6·45	8·50	35	26	26	185	265	482
4	4·30	8·48	62	63	69	262	372	875
5	4·70	8·50	38	32	31	96	151	215
6	4·30	8·38	36	30	30	96	160	275
7	4·50	8·36	42	36	37	228	391	1247
8	5·80	8·47	56	54	57	196	261	635
9	4·65	8·35	78	82	98	222	290	791
10	5·50	8·42	100	111	143	630	1158	1800
11	5·30	8·36	81	89	116	329	617	1800
12	4·70	8·45	99	103	119	345	612	1800
Average		8·44	68	72	90	265	336	900

TABLE VI

Rennet test with cow milk and tonned cow milk (roller dried skim-milk powder)

(Coagulation time in seconds)

Serial Number	Fat of original raw milk	S. N. F. of original raw milk	Raw samples: Original milk, Percentage adulteration 0	Original milk, 30	Original milk, 50	Tonned milk, 0	Tonned milk, 30	Tonned milk, 50
1	4·30	8·53	62	63	69	148	233	280
2	5·95	8·56	91	106	142	780	1590	1800
3	4·40	8·53	52	49	48	184	203	322
4	4·70	8·56	38	32	31	63	64	76
5	4·30	8·56	36	30	30	56	58	61
6	4·50	8·47	42	36	37	255	444	1130
7	4·65	8·61	78	82	98	281	436	1040
8	5·30	8·42	81	89	116	385	745	1800
9	5·30	8·50	75	75	86	405	646	1410
10	5·20	8·41	69	70	89	298	422	1036
11	3·90	8·49	72	77	90	270	372	552
12	4·10	8·56	74	78	79	255	385	494
Average		8·52	64	66	76	282	466	833

The following is a summary of the results given above :

(*A.*) *Buffalo milk tonned with spray-dried skim milk powder* (*Table I*)

(i) With raw buffalo milk, the time of coagulation on dilution with distilled water tends to decrease slightly with 15 and 30 per cent dilution and increases by about 7 per cent with 50 per cent dilution.

(ii) Under identical conditions of dilution, the time of coagulation of tonned milk increases as shown by the following average figures :

	Average time of coagulation (seconds)	Minimum and maximum limits (seconds)		Percentage increase over original	Percentage increase over tonned milk
Original buffalo milk	53	35	98	..	..
Tonned ,, ,, ...	85	53	129	60·4	..
Tonned milk diluted 15 per cent	91	55	133	71·7	5·8
,, ,, ,, 30 per cent	96	56	191	81·1	12·9
,, ,, ,, 50 per cent	136	84	297	156·6	60·0
Milk powder solution	379	285	650	..	..

(iii) Boiled buffalo milk gives an average value of 148 seconds for coagulation (minimum 79 maximum 257) ; *i.e.*, an increase of 179 per cent over the value for raw milk.

(iv) The increase in time of coagulation of boiled buffalo milk with different dilutions were as follows :

Dilution	Average time of coagulation (seconds)	Minimum and maximum limits (seconds)		Percentage increase over boiled milk
15 per cent	160	84	257	8·1
30 per cent	202	89	453	36·5

(v) Boiled tonned milk shows the following changes :

Dilution	Average time of coagulation (seconds)	Minimum and maximum limits (seconds)		Percentage increase over boiled milk	Percentage increase over tonned boiled milk
Boiled tonned milk	210	110	323	41·9	..
,, ,, ,, diluted 15 per cent	254	112	403	71·6	20·9
,, ,, ,, ,, 30 per cent	402	134	677	171·6	91·4

(B) *Cow milk tonned with spray-dried milk powder* (*Table II*)

(i) Raw cow milk shows a distinct increase in time of coagulation with different dilutions as follows :

Dilution	Average time of coagulation	Percentage increase over original milk
15 per cent	89	3·5
30 ,,	101	17·5
50 ,,	154	79·1

(ii) Under identical conditions of dilutions, the time of coagulation of tonned cow milk gave the following results :

	Average time of coagulation	Minimum and maximum limits		Percentage increase over original milk	Percentage increase over tonned milk
Tonned milk	116	45	180	34·9	..
,, ,, diluted 15 per cent	115	45	223	33·7	0
,, ,, ,, 30 per cent	168	46	357	95·3	46·1
,, ,, ,, 50 per cent	284	72	425	230·2	147·0

(iii) Boiled samples take on an average 288 seconds for coagulation, *i.e.*, an increase of nearly 335 per cent over raw milk. The corresponding figure for buffalo milk as mentioned before was 179 per cent.

(iv) The increase in time of coagulation of boiled cow milk with different dilutions were as follows :

	Average time of coagulation	Minimum and maximum limits		Percentage increase over boiled milk
Boiled cow milk	288	145	522	..
,, ,, ,, diluted 15 per cent	477	166	1027	65·6
,, ,, ,, ,, 30 per cent	951	194	1800	230·2

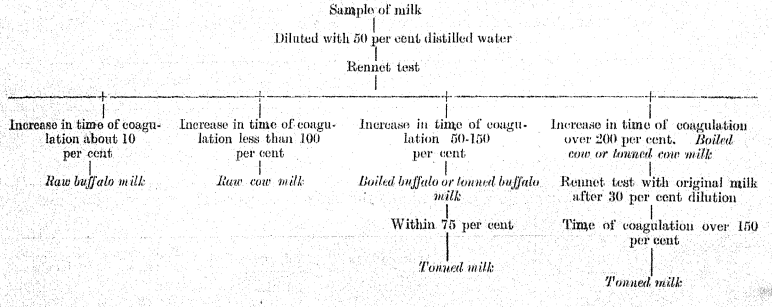
(v) Boiled tonned milk show the following changes :

	Average time of coagulation	Minimum and maximum limits		Percentage increase over boiled milk	Percentage increase over boiled tonned milk
Boiled tonned milk	367	137	667	27·4	..
,, ,, ,, diluted 15 per cent . .	646	172	1350	124·3	76·0
,, ,, ,, ,, 30 per cent . .	1124	244	1920	290·2	206·3

(C) *Cow and buffalo milk tonned with rollerdried milk powders* (*Tables III-VI*)

From the results it is seen that the addition of these particulars brands of roller dried powders increases the time of coagulation to a very great degree and there is no ambiguity in detecting tonned milk.

On the basis of the above results a tentative scheme for differentiating genuine, tonned and boiled milk is given below—



This scheme can be still more simplified if it is assumed that under market conditions the analyst will have to deal with either raw cow, buffalo or tonned milk. In that case the rennet test is carried out with original milk and of the same milk after 50 per cent dilution with distilled water. The interpretation of results will be as follows :—

1. Change in time of coagulation not exceeding 10 per cent . . . . . Buffalo milk
2. Increase in time of coagulation between 20-50 per cent . . . . . Tonned buffalo milk
3. Increase in time of coagulation between 75-100 per cent . . . . . Cow milk
4. Increase in time of coagulation of 200 per cent and over . . . . . Tonned cow milk

(iii) *Estimation of total Residue.* A quantity of milk 40 ml. were centrifuged in a weighed tube for 25 minutes at a speed of 3,000 r.p.m. The supernatent liquid, including the fat layer, were decanted off without disturbing the sediment at the bottom and drained for 30 seconds. The sides were wiped carefully with wet cotton-wool to remove any fat sticking to them. The residue was dried, cooled, weighed and results expressed as gm. 100 ml.

Preliminary trials showed that when working with wet residue concordant results were not obtained and it was necessary to dry the residue to a constant weight.

The results are given in Tables VII-X.

Table VII

Dry residue in tonned buffalo milk (spray-dried skim milk powder) expressed in gm./ 100 ml.

Serial No.	Raw samples			Boiled samples		
	Percentage dry residue			Percentage dry residue		
	Original milk	Tonned milk	Milk powder milk used for tonning	Original milk	Tonned milk	Milk powder milk used for tonning
1	0·1062	0·1930	0·4160	0·1095	0·1365	0·2010
2	0·0890	0·2270	0·3440	0·0660	0·1460	0·1570
3	0·0795	0·1805	0·2870	..	..	..
4	0·1147	0·2320	0·3615	0·1225	0·1775	0·1955
5	0·0600	0·2120	0·3345	0·0535	0·1220	0·1515
6	0·0937	0·2367	0·3807	0·1157	0·1430	0·1515
7	0·1160	0·2635	0·3150	0·1055	0·1130	0·1690
8	0·0560	0·1877	0·3590	..	..	..
9	0·0630	0·2010	0·3335	0·1160	0·1500	0·1920
10	0·1195	0·2182	0·3941	0·1202	0·1240	0·1527
11	0·0700	0·2530	0·3170	0·1520	0·2545	0·2440
12	0·0870	0·1967	0·3957	0·0935	0·1500	0·2005
13	0·1302	0·2250	0·3295	0·0975	0·1675	0·2125
14	0·1040	0·2015	0·3015	0·0960	0·1990	0·2085
15	0·0895	0·1780	0·3847	0·0860	0·1390	0·1635
16	0·0700	0·1855	0·3595	0·1120	0·1195	0·1780
17	0 0765	0·1805	0·3375	0·1505	0·0687	0·2192
18	0·0920	0·2080	0·2180	0·1060	0·057)	0·1740
19	0·0475	0·1515	0·2727	0·0970	0·1450	0·1695
20	0·0840	0·2305	0·2780	0·0740	0·1412	0·1687
21	0·0740	0·1832	0·3960	0·1602	0·2259	0·2768
22	0·0830	0·1235	0·3128	..	..	..
23	0·0910	0·1630	0·2820	0·1235	0·1510	0·2015
24	0·0437	0·2172	0·4170	0·0967	0·1185	0·1457
25	0·1230	0·1700		0·1477	0·1510	
26	0·0525	0·1637		0·1150	0·1282	
27	0·1050	0·1662		0·0525	0·0765	
28	0·0560	0·0907		0·1050	0·1030	
29	0·0980	0·1470		0·0965	0·1175	
30	0·0690	0·1185		0·1005	0·1890	
31	0·0912	0·1262		0·0657	0·1130	
32	0·0875	0·1372		0·0900	0·0985	
33	0·0950	0·1645		0·0650	0·1050	
34	0·0785	0·1447		0·1022	0·1300	
35	0·0890	0·1990		0·0945	0·1275	
36	0·0730	0·1662		0·1122	0·1482	
37	0·1190	0·1827		0·1105	0·1730	
38	0·1120	0·1880		0·1125	0·1580	
39	0·0807	0·1455		0·1022	0·1222	
40	0·0802	0·1637		0·1060	0·0144	
41	0·0572	0·1177		0·1005	0·1415	
42	0·0715	0·1400		0·1090	0·1380	
43	0·0522	0·1352		0·1012	0·1272	
44	0·0512	0·1125		0·1035	0·1230	
Average	0·0814	0·1778	0·3428	0·1037	0·1325	0·1872

TABLE VIII

Dry residue in tonned cow milk (spray-dried skim-milk powder) expressed in gm./100 ml.

Serial No.	Raw samples			Boiled samples		
	Original milk	Tonned milk	Milk powder milk used for tonning	Original milk	Tonned milk	Milk powder milk used for tonning
1	0·0650	0·1250	0·3345	0·0545	0·0960	0·1515
2	0·0417	0·1150	0·3807	0·0475	0·1090	0·1515
3	0·0980	0·1140	0·3150	0·0495	0·0885	0·1690
4	0·0270	0·0790	0·3590	..	..	..
5	0·0690	0·1560	0·3335	0·0740	0·0870	0·1920
6	0·0630	0·1380	0·3941	0·0610	0·1097	0·1527
7	0·0565	0·1490	0·3170	0·0920	0·1350	0·2440
8	0·0480	0·1410	0·3957	0·0725	0·1155	3·2005
9	0·0645	0·1311	0·3295	0·0657	0·1250	0·2125
10	0·0805	0·1030	0·3015	0·1475	0·1180	0·2085
11	0·0420	0·1260	0·3847	0·0512	0·1192	0·1635
12	0·0580	0·1740	0·3595	0·1200	0·1300	0·1780
13	0·0420	0·1535	0·3375	0·1920	0·1700	0·1292
14	0·0370	0·0727	0·2727	0·0920	0·1310	0·1695
15	0·0775	0·1140	0·2795	0·0512	0·0962	0·2127
16	0·0503	0·0930	0·3128	..	..	..
17	0·0882	0·1110	0·2820	0·0685	0·0985	0·2015
18	0·0797	0·2115	0·4170	0·0370	0·0975	0·1457
19	0·1045	0·1722				
20	0·0542	0·1412				
21	0·0502	0·1202				
22	0·0655	0·1152				
23	0·0705	0·1150				
24	0·0690	0·1450				
25	0·0602	0·0937				
26	0·0547	0·0982				
27	0·0670	0·1125				
28	0·0515	0·1262				
29	0·0990	0·1455				
30	0·0582	0·0962				
31	0·0697	0·1520				
32	0·0765	0·1190				
33	0·0677	0·1232				
34	0·0505	0·1230				
35	0·0680	0·1100				
36	0·0397	0·1247				
37	0·0557	0·1265				
Average	0·0605	0·1261	0·3392	0·0797	0·1141	0·1857

TABLE IX

Dry residue in tonned milk using roller dried whole-milk powder expressed in gm./100 ml.

Serial No.	Raw Buffalo milk		Boiled Buffalo milk		Raw Cow milk	
	Original	Tonned	Original	Tonned	Original	Tonned
1	0·0845	0·5129	..	..	0·0507	0·6680
2	0·0795	0·7712	0·0735	0·2690	0·0697	0·5150
3	0·0660	0·7750	0·0815	0·6215	0·0815	0·5831
4	0·0800	0·5370	0·0900	0·2300	0·0587	0·5555
5	0·0575	0·7650	0·1660	0·2135	0·0540	0·2865
6	0·0730	0·3800	0·2447	0·2395	0·0720	0·4207
7	0·0702	0·6525	0·1685	0·1817	0·0700	0·2835
8	0·0550	0·4027	0·1195	0·2425	0·0572	0·1862
9	0·0750	0·3444	0·1730	0·4020	0·0482	0·8610
10	0·0700	0·7290	0·1175	0·3757	0·0635	0·2600
11	0·0140	0·4845	0·1110	0·2280	0·0512	0·6540
12	0·0698	0·5335	0·0965	0·3120	0·0670	0·3125
Average	0·0662	0·5739	0·1310	0·3014	0·0620	0·4655

Table X

Dry residue in tonned milk using roller dried skim-milk powder expressed in gm./100 ml.

Serial No.	Raw buffalo milk		Boiled buffalo milk		Raw cow milk	
	Original	Tonned	Original	Tonned	Original	Tonned
1	0·0800	0·3060	0·0900	0·2145	0·0670	0·1910
2	0·0825	0·2812	..	..	0·0410	0·3292
3	0·0637	0·3255	0·1195	0·2937	0·0850	0·7340
4	0·0525	0·4400	0·1073	0·3050	0·0566	1·1320
5	0·0945	1·0530	0·1127	0·2077	0·0720	0·3037
6	0·0730	0·2940	0·1275	0·2447	0·0700	0·2835
7	0·0702	0·6525	0·1685	0·1817	0·1070	0·3925
8	0·0625	0·3660	0·1065	0·2360	0·0482	0·3620
9	0·0750	0·3675	0·1845	0·4020	0·0635	0·2600
10	0·0700	0·7000	0·1115	0·3941	0·0615	0·5120
11	0·0660	0·6620	0·1065	0·2415	0·0557	0·4062
12	0·0625	0·4216	0·1125	0·2368	0·0612	0·5265
Average	0·0710	0·4891	0·1215	0·2689	0·0616	0·4527

It was found that fresh milk samples give values much below 0·10 per cent dry residue in a large majority of cases and the average varies from 0·060 to 0·081 per cent. There was a great variation in results obtained from the same class of animals on different days and hence it was not possible to differentiate between cow and buffalo milk from individual figures. It may further be added that the studies reported here were carried out with farm produced milk and it is quite likely that market samples will give higher values for dry residue than those recorded. Compared to raw cow and buffalo milk, milk powder solutions give much higher values. The average for spray dried skim-milk powder was 0·34 per cent. Tonning raw milk invariably gives a higher figure. When roller dried milk powders are used tonning can be detected without any difficulty as the percentage of dry residue increases from less than 0·05 per cent to nearly 0·5 per cent.

When spray dried powder is used the percentage of dry residue does increase but the demarcation is not so sharp. For guidance it is suggested that if the percentage of residue is greater than 0·11 per cent the possibility of the sample being tonned may be tentatively assumed and confirmed by the following further tests :

(*a*) The dry residue may be estimated on the sample after it has been boiled. It has been found that milk samples give a higher percentage of residue after boiling. On the contrary tonned milk (as well as spray dried skim milk powder) shows the reverse, *i.e.*, on boiling the amount of residue usually decreases, probably due to the fact that on boiling some more of the residue goes into solution.

(*b*) The dry residue is subjected to the xantho-protein reaction. The residue is dissolved in 2·5 ml. concentrated nitric acid, diluted with 5 ml. of water and then 2·5 ml. liquor ammonia added. It was observed that the residue from pure milk gives a solution with a yellow colour, milk powder residue gives a deep orange solution, while tonned milk samples give a colour varying from light orange to orange. The results are given in Table XI.

TABLE XI

Xanthoprotein reaction with dry residue

Serial No.	Buffalo milk tonned with spray dried skim-milk powder						Cow milk tonned with spray dried skim-milk powder			
	Raw samples			Boiled samples			Raw samples		Boiled samples	
	Original	Tonned	Milk powder milk used for tonning	Original	Tonned	Milk powder milk used for tonning	Original	Tonned	Original	Tonned
1	Y	+	++++	++	+++	++++	+	+++	++	+++
2	Y	+	++++	Y	+++	++++	Y	+++	Y	++
3	Y	+	++++	—	—	—	Y	+++	—	—
4	Y	+	++++	Y	+++	++++	Y	+++	Y	+
5	Y	+	++++	Y	+	++++	Y	+++	Y	+
6	Y	+	++++	Y	+	++++	Y	+++	Y	++
7	Y	+	++++	Y	+	++++	Y	+++	Y	+
8	Y	+	++++	—	—	—	Y	++	—	—
9	Y	+	++++	Y	+++	++++	Y	++	Y	+
10	Y	+	++++	Y	++	++++	Y	++	Y	+
11	Y	+	++++	Y	++	++++	Y	+++	Y	+
12	Y	++	++++	+	++	++++	Y	++	Y	+
13	Y	++	++++	Y	++	++++	Y	++	Y	+
14	.	+++	++++	+	+++	++++	Y	+++	Y	++
15	Y	+++	++++	+	+++	++++	Y	++	Y	+
16	Y	+++	++++	Y	+++	++++	Y	++	Y	++
17	Y	+++	++++	+	+++	++++	Y	++	Y	++
18	+	++	++++	Y	++	++++	Y	++	Y	++
19	+	++	++++	Y	++	++++	—	++	—	+
20	Y	+	++++	—	—	—	Y	++	—	—
21	Y	+	++++	+	+	++++	Y	++	Y	++
22	Y	++	++++	++	+++	++++	Y	++	Y	++
23	Y	+	—	Y	++	—	Y	+		
24	Y	+	—	Y	+	—	Y	+		
25	Y	+	—	++	+	—	Y	++		
26	Y	+	—	+	+++	—	Y	+		
27	Y	+	—	Y	+	—	Y	+++		
28	Y	++	—	Y	+	—	Y	+		
29	Y	+	—	Y	++	—	Y	+		
30	Y	+	—	Y	+	—	+	+++		
31	Y	+	—	Y	+	—	Y	+		
32	Y	+	—	Y	+	—	Y	+		
33	Y	Y	—	Y	+	—	Y	+		
34	Y	+	—	Y	+	—	Y	+		
35	Y	+	—	Y	+	—	Y	+		
36	+	+++	—	Y	+	—	Y	++		
37	Y	+	—	Y	Y	—	Y	Y		
38	Y	+	—	Y	+	—	Y	++		
39	Y	+	—	Y	+	—	Y	++		
40	Y	++	—	Y	+	—	Y	+		

Y=Yellow
+=Yellowish orange
++=Light orange
+++=Orange
++++=Deep orange
—=Test not carried out

This test formed a useful and simple means of distinguishing pure and tonned milk. In the Table XI results of experiments with spray dried skim milk powder alone are given. However, these conclusions have been confirmed when using roller dried powers also.

(iv) *Other tests tried.* Besides the ones indicated above, original and tonned milk samples were subjected to the following tests:

a. Freezing point determination
b. Microscopic study
c. Methylene blue reduction test
d. Resazurin test, and
e. Estimation of total minerals

None of these tests helped to clearly differentiate between various samples of milk and hence detailed results are not given here.

SUMMARY

The following nine methods were tried for differentiating genuine milk from tonned milk:

(1) Stability to heat
(2) Coagulation with rennet after dilution with water
(3) Estimation of dry residue
(4) Xanthoprotein reaction with dry residue
(5) Freezing point
(6) Microscopic study
(7) Methylene blue reduction test
(8) Resazurin test, and
(9) Estimation of total minerals

Studies have been carried out using spray dried skim-milk powder, roller dried skim-milk powder and roller dried whole milk powder.

Amongst the tests tried microscopic study, methylene blue test, resazurin test, freezing point and estimation of total minerals have proved of little value.

The odour of samples after incubation for four hours at 37°C. provides the simplest and a very reliable test. Incubation is carried out after adding a drop of formalin to suppress the development of lactic acid odour. This test can be done under house-hold conditions.

The rennet test after diluting the milk sample with distilled water is another simple test and the results can be obtained in a short time. Tonned milk takes a much longer time to coagulate. Cow and buffalo milk are found to differ to some extent in their behaviour towards rennet. Cow milk takes a longer time and also the time of coagulation markedly increases on dilution with water.

The estimation of dry residue is another test which will prove useful. It is likely that the figures for dry residue obtained in the present studies may require some modification when working with market samples. The results of this test are further confirmed by the xanthoprotein reaction with the dry residue. Pure milk gives a yellowish coloured solution, whilst tonned milk samples give an orange colour.

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STUDIES ON THE HAEMOTOLOGY OF DOGS IN HEALTH AND WHEN INJECTED WITH *BABESIA GIBSONI* (PATTON 1910)

By B. M. PRASAD, Imperial Veterinary Research Institute, Mukteswar

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THE purpose of the present investigation was to obtain precise data on the total red and white cell counts, the differential white cell counts and the haemoglobin content of the blood of dogs in health and when suffering from piroplasmosis due to infection with *Babesia gibsoni*.

MATERIAL AND METHODS

Three healthy pariah dogs were used. To obtain the normal blood picture, seventeen counts were made on ten other healthy dogs and the results obtained were more or less in agreement with those obtained with the three original dogs at different periods prior to infective inoculation. The dogs were obtained from Almora in the Kumaon hills and their age ranged from two to four years and body weight from twentyfive to tairty pounds. Two of the three animals were artificially infected with a laboratory strain of *B. gibsoni* and the other served as a control. Towards the close of the experiment this dog, too, was given a dose of infected blood to see if it was susceptible to the disease and a fatal piroplasmosis resulted. The injections were given subcutaneously with the idea that the disease might develop as under natural conditions; the disease is known to be transmitted from one animal to another by the tick Haemaphysalis bispinosa. The animals were housed together and kept on an uniform diet consisting of $\frac{1}{2}$ lb. of cooked beef and *chapattis* given in one meal in the afternoon; but during the acute stage of the disease the affected dogs refused solid food and were then given either milk or soup. The shed was a hundred yards from the laboratory. this distance was traversed twice daily by the dogs and constituted their daily exercise. When the illness was advanced, they were unfit to walk, and remained indoors with the control animals. Blood samples were always drawn at the fixed time of day (10 A.M.)

Both the dogs took the infection and it is interesting to note that while in one, the disease was fatal in about 16 days after the first appearance of the parasites in the blood, in the other, it was chronic and the animal apparently recovered.

All samples of blood were collected from the marginal ear-vein or the external Sephana vein, as between these two sites there was no difference in the blood picture. The area where the puncture was to be made was cleaned with cotton wool and alcohol, rubbing being continued for about one minute. When the alcohol had evaporated, a puncture was quickly made with a thin sterilized hypodermic needle and after rejecting the first two or three drops, the blood was taken up in a capillary pipette direct from the puncture.

For red cell counts the blood was diluted 200 times in a potain pipette (0·5—101). The pipette selected for this purpose gave almost identical counts with the same sample of blood. During the time the two experimental animals showed signs of severe anaemia, it was necessary, however, to make the dilution 1 to 100 instead of 1 to 200. Toisson's fluid was used for diluting purposes. A Thoma (Hawskley) haemocytometer was used and in each case a hundred squares were examined.

Before counting, the white cells were also diluted in potain pipettes at 1 to 11 dilution. It was essential to use a 4 per cent acetic acid solution as the diluting fluid, since if used in weaker strength than this, results were unsatisfactory. Thoma (Hawskley), having only one large ruled square instead of nine as in others, is not very suitable for white cell counts. However its use makes it necessary to examine at least four-fresh preparations to obtain an average of counts.

Determinations of haemoglobin were made by the Sahli method. Although the advantages and defects of this have been frequently discussed, the results obtained with it were for practical purposes satisfactory.

For differential leucocyte counts ordinary clear glass slides were used. For this purpose smears were prepared from a puncture at the tip of the ear. The hair was cut close and the part sterilized with 70 per cent alcohol; when this had dried, a piano touch like stroke with a hypodermic needle

was made and the first drop that oozed out without squeezing was taken up on a slide and spread over another as rapidly and uniformly as possible. Giemsa stain gave good results. Immature cells of lymphoblastic and myeloblastic origin were differentiated by the oxydase reaction in freshly-prepared-blood smears. For this purpose the method recommended by Piney [1931] was generally followed.

Films, immediately after air-drying were put in a glass stoppered jar containing 2 per cent osmic acid and allowed to stand for 30 seconds for complete fixation. They were then washed thoroughly with distilled water and stained for 10 minutes with a mixture of equal parts of 1 per cent solutions of alpha-naphthol and paraphenylenediamine. They were then washed in distilled water and mounted without drying in water glass, oxydase granules being stained blue. For the actual content of the leucocytes the smears were mounted and examined by the method known as the 'Four field meander' technique, and Arneth Index was taken for neutrophiles.

Experimental

From Table I it may be observed that compared with figures reported by various workers elsewhere the average erythrocyte count per c.mm. of blood in the experimental dogs is within the normal range of variation.

Table I

Blood-counts in healthy dogs

	Erythro-Leucocytes cytes (millions)		Differential counts				
			Lym.	Neut.	Eosins.	Mono.	Baso.
[1]. Prasad	5·706	12370	22·5	61·9	9·3	5·9	0·4
[2]. Dukes (1935) . . .	6·160	12600	25	57	10	8	0·0
[3]. Kohanawa (1928) . .	6·998	17050	13·2	77·4	3·3	6·1	—
[4]. Gaiger & Davies (1938) .	6·1	13000	21	64	9	6	—
[5]. Nicols (1935) . . .	7·16	11068	—	—	—	—	—

While the count agrees closely with those of Duke's [1930], Gaiger and Davie [1938], it is slightly below the figures reported by Kohanawa [1938] and Nichols [1935]. Similarly, the total white cell counts and the differential counts differ to a certain extent from the figures mentioned by Kohanawa, but are in close agreement with the figures reported by other workers.

Haemoglobin was estimated to be about 13.95 gm. per 100 c.c. of blood by weight. Figures of other workers are not available for comparison.

General characters of the blood changes in infected dogs

The outstanding change in the blood of dogs infected with *B. gibsoni* is that of severe anaemia. That there is a rapid fall in the red cell count as soon as the disease is fully established will be seen from the figures for Dog No. 136, Table II.

The extreme anaemic condition apparently results in part from the disintegration of red corpuscles heavily laden with parasites but chiefly, from diminished activity of cell regenerating organs. The first pathological appearance noticed is achromia, the red cells staining faintly owing to the general lack of haemoglobin. This feature is constant and is soon followed by a series of other changes, such as slight anisocytosis associated with polychromatophilia, in which a varying number of immature cells appear and which being deficient in haemoglobin take a dirty blue tint. The proportion of such cell ranges from 4 to 8 per cent depending upon the severity of the disease. Later, it is not uncommon to find nucleated red cells and in very severe cases anisocytosis becomes very marked. Punctate basophilia and punctate degeneration of red cells or nucleated red cells, so commonly observed in other species of animals in similar conditions are of rare occurrence in dogs.

White cells. It will be observed from Table II that with the appearance of first symptoms, the leucocytes begin to increase in number and reach a peak in about a week's time. From a normal

TABLE II

Blood picture of experimental dogs

Date (1938)	No. of observations	h-b. present	White cells in thousands	Red cells in millions	Differential Counts: Neutrophil 1	2	3	4	5	Total	Lymphocyte	Eosinophiles	Monocyte	Basophils	Remarks
					Dog No. 136										
				5·9	3·8	21·0	23·6	11·6	2·2	61					
29-3 to 6-4	6	81·0	12·8		(Infected with virulent blood on 8.4.38).						22·5	10·0	6·3	0·3	
				5·7	4·2	20·0	24·6	9·5	2·0	62·8					
				3·9	14·8	23·7	23·0	10·0	2·8	74·3					
8-4 to 18-4	6	76·2	14·8	1·8	16·2	12·7	12·1	6·0	1·4	48	20·8	9·0	6·0	0·2	
20-4 to 30-4	7	42·0	53·3								15·0	4·0	7·0	0	
2-5 to 12-5	11	13·1	11·9								17·0	4·0	30·4	0	Died
					Dog No. 138										
1-4 to 6-4	4	80·7	12·7	5·7	3·8	21·5	24·0	11·0	1·0	62·5	22·0	9·5	5·5	0·2	
					(Infected with virulent blood on 8.4.38)										
8-4 to 18-4	6	78·3	14·2	5·8	4·2	20·3	25·6	11·8	1·5	62	22·5	9·3	6·0	0·1	
20-4 to 30-4	6	52·1	34·7	4·7	11·5	19·0	25·2	12·5	3·2	69	18·0	7·1	6·0	0	
1-5 to 11-5	11	12·5	34·7	2·7	13·6	17·7	17·1	6·6	1·4	54·3	19·0	4·7	5·2	0	
12-5 to 27-5	16	20·0	13·6	2·1	9·7	15·5	21·7	8·0	0·8	56	20·2	5·0	18·4	0	
					Dog No. 137 (Control)										
9-3 to 6-4	6	81·3	13·6	5·6	3·5	19·2	23·0	11·5	2·0	60	21·0	13·0	6·0	10	
8-4 to 18-4	6	81·5	13·5	5·8	3·8	17·1	25·1	12·8	3·8	59·8	23·0	9·6	6·8	0·6	
20-4 to 30-4	6	77·1	18·8	5·6	4·3	17·5	25·1	13·1	2·5	62·3	21·5	9·5	4·8	0·1	
2-5 to 12-5	6	81·1	12·6	5·7	4·5	18·3	23·0	12·5	2·6	61·3	23·0	9·6	5·2	0·2	
14-5 to 26-5	7	81·1	12·2	6·1	4·2	15·3	23·0	13·1	2·0	61·4	22·3	10·0	6·3	0·1	

N.B.—Figures show the mean value of the different constituents over the period stated.

of 12570 per c.mm. a rise to 82140 per c.mm. of blood has been noticed. The rise in the number of leucocytes marks the beginning of the reaction, but the number gradually returns to normal level or even slightly below it. During the period of marked leucocytosis the percentage of various types of cells, although not adversely affected, decrease in proportion to increase of neutrophiles which is about 20 per cent of the normal, with a shift to the left. Later, there is an increase in the number of large mono-nuclear to the extent of about 28 per cent on an average, with the reduction in the number of other cells. A few cells of both myeloblastic and lymphoblastic origin have not infrequently been observed in the differential count. In the recovering animal the percentage of different types of cells gradually returns to normal but in cases of fatal infection monocytosis persists.

Red cells. There is a marked decrease in the number of red cells. From a normal of about five millions prior to inoculation, a decline to about one million per c.mm. of blood has been found. The decrease in the number of cells becomes noticeable after a lapse of fortnight, although there may not be any appreciable rise in the temperature, and this decrease may occur even before the parasites have appeared in the peripheral circulation. In rapidly progressive cases the fall in the red cell count may, however, be observed earlier. This statement is based on sporadic cases of disease in dogs not included in the present paper. Table II (Dog Number 138) shows that the process of restoration of the number of red cells to normal is slow.

Haemoglobin. An examination of Table II shows a marked variation in the blood haemoglobin content during health and disease; from a normal of about 14 gm. haemoglobin per 100 c.c. of blood there is a reduction to about 2·18 gm. The figures further show that during the crisis of the disease the available haemoglobin is functioning at its maximum capacity. The severe drop in haemoglobin lowers the oxygen-carrying capacity of the blood to a point at which it may be insufficient to maintain life, hence distressing respiratory efforts are manifested in advanced clinical cases.

SUMMARY

This paper records the results of studies made to determine the total red and white cell counts, the differential white cell counts, and the haemoglobin content of the blood of dogs during health and during piroplasmosis (*B. gibsoni* infection) of there apparently healthy dogs between two to four years of age, two of which were infected artificially with the parasites and the third was used as a control. Morphological studies and haemoglobin estimations were made of the blood of these animals before infection and also during the disease. In the two infected dogs the readings did not change significantly during the incubation period.

For comparative purposes seventeen (17) counts were made on the blood of 10 apparently healthy pariah dogs with results similar to those obtained in the case of the experimental dogs before infection was established.

In the two infected dogs the red cell count fell to about one million per c.mm. during the disease. The white cell count increased to a peak at nearly 80,000 per c.mm. and this was followed by a gradual decrease to almost normal level, irrespective of whether the disease ended in death or recovery. The differential white cell count showed during a period of marked leucocytosis a mild neutrophilia, slight lymphopenia, eosinopenia, and a shift to the left of the neutrophiles and this was followed by a moderate monocytosis. During the disease the average haemoglobin content dropped to 12·5 per cent from normal.

The outstanding features of *B. gibsoni* infection in dogs are the decrease in total red cell count and the marked fall in haemoglobin due to a certain extent to rapid destruction of the red cells, but more to the failure of formative tissues.

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STUDY ON THE VITAMIN A ACTIVITY OF CAROTENE IN GREEN FODDER

By B. C. Roy Sarkar and K. C. Sen, Imperial Veterinary Research Institute, Izatnagar, and Imperial Dairy Research Institute, Bangalore, India

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In some recent papers, Seshan and Sen (1942 a.b.c.d) described a modified technique for extracting carotene from different feeding stuffs and utilized it for the study of carotene in plants including some balance experiments with ruminants. It was pointed out by the authors that the term carotene was used in the ordinary sense denoting the epiphasic pigment, and no chromatographic study was made of the time. As such, the carotene figures for materials like old stored hay straw, silage and cattle faeces were liable to revision because of the production of non-carotene pigments not removable by phase separation with 90 per cent methyl alcohol used in the method, but it was felt that no substantial error was likely to be caused in the case of green fodders. It was, however, decided to check up the method by simultaneously carrying out a biological and chromatographic assay of the carotene contained in the feeding stuffs. The results obtained are being presented below, and based on these, the Seshan-Sen method of extraction of carotene has been combined with chromatographic adsorption for the routine analysis of vitamin A-active carotene in feeding stuffs. While these data were being written up, Austin and Shipton [1944] published a critical examination of the methods of determining carotene and came to the conclusion that, of the variety of methods designed for the extraction of carotene, that proposed by Seshen and Sen was the most satisfactory, being decidedly more efficient and more widely applicable than the process recommended by Peterson, Hughes and Freeman [1937]. Austin and Shipton have suggested the use of 'heavy' magnesia for the chromatographic separation of non-carotene pigments in conjunction with slightly modified Seshan-Sen method of extraction.

Although a fair amount of work has been done by different investigators on the determination of carotene for evaluating theoretically the vitamin A potency of stock feeds, there are comparatively few data on the relation between the carotene content and the vitamin A potency of those materials as measured biologically. Woods, Shaw, Atkeson and Johnson (1932), Woods, Atkeson, Wellhonsen and Johnson (1935a). Woods, Atkeson, Shaw, Slater and Johnson (1935b), Woods, Atkeson, Slater, Arndt, and Johnson (1935c) and Archibald, Bennett and Pitchie (1943) determined the biological potency of certain grasses, but their carotene content was not simultaneously estimated. The work of Taylore, Russell and Bender (1939) on the carotene content and vitamin A potency of different types of silage, and that of Hodgson, Murer and Knott [1939] on green, dehydrated and sun cured pea vines and pea vine silage, etc., may also be mentioned, although some of the results obtained are of questionable value. In the case of green vegetables and other plant foods, some work by different workers is on record. Felice and Fellers [1937] obtained a factor 1·7 for converting the carotene values of fresh, frozen, canned and dehydrated samples of spinach into the vitamin A potency. Gorter and Spruyt (1939) reported that some foodstuffs were found to contain less provitamin A by the rat test than that obtained by chemical analysis. Stimson, Tressler and Maynard [1939] found the ratio of vitamin A potency to carotene to be 1·5 in the case of fresh and frosted peas. Smith and Otis (1941) pointed out the ralative inefficiency of yellow vegetables, notably carrot when compared with green sources. Greaves [1942] observed that carotene of vegetables seems to be less effective biologically than vitamin A and the vitamin A activity per unit of carotene was much greater in spinach, water cress and alfalfa than in carrots. Zimmerman, Tressler and Maynard [1940, 1941] found that much of the pigment of Golden Cross Bantam Sweet Corn was not carotene, while the vitamin A activity of asparagus was solely due to β-carotene and that of green lima bean due to two thirds β-carotene one-third α-carotene.

To explain the discrepancy between the chemically determined carotene and its biological activity and the superiority of carotene from one type of material over that from another, it is desirable to know whether carotene estimated chemically is biologically as active as β-carotene, and if not, to determine its purity; secondly, how far the ingested carotene is absorbed and utilized in the system. For the elucidation of these points, the following series of experiments were conducted.

Experimental

(1) *Relation between the chemically determined carotene and its biological potency as assayed against standard carotene.*

The experimental part consisted mainly of (a) determination of the carotene content of some green fodders by the Seshan-Sen technique, and (b) the bio-assay of the same materials by using Coward's curative methods [1938] with albino rats. The basal diet for the rats consisted of vitamin A-free casein 18, dried brewer's yeast 10, dextrinized starch 63, purified coconut oil 5 and MaCollum, Simmonds and Pitz salt mixture 4 parts respectively. Ten units of calciferel in coconut oil solution were given per *os* to each animal once a week. Five to ten animals were used in each group (negative control, positive control, and test group). The standard of reference was a sample of B.D.H. carotene, the purity of which was determined spectrographically [Morton, 1942] and a solution containing 10 mg. of true carotene in 50 c.c. of coconut or olive oil was prepared using 5 mg. of hydroquinone as antioxidant, and this was kept in the dark at a low temperature. Carotene was stable in both these oils for a long period and this served as stock solution from which weekly dilutions were made for administering carotene to the positive control animals daily. The methods of dosing the test material was first to dry quickly the green fodders in a current of air at a temperature not exceeding 62°C. It was found that the carotene content was not affected if the whole plant or leaves were dried in this way, but there was some loss if the plants were chopped up so as to disintegrate some of the material and expose the contents to atmospheric oxidation. The dry material was then ground to a fine powder, analysed for its carotene content, and then mixed with a small amount of vitamin A-free casein and fed to individual rats quantitatively before the basal ration was given to them. This ensured a quantitative intake of the fodder carotene. In the case of barley extract, the carotene was extracted by petroleum ether after the usual alkali alcohol digestion, evaporated to dryness, dissolved in a small amount of oil and then fed to experimental animals. The growth experiments were conducted for 4 weeks after the test animals reached a constant weight on the vitamin A-free basal ration. The dosing of the positive control and the test group of animals was so arranged by preliminary experiments that almost equal growth was obtained during the assay. In Table I

Table I

Vitamin A potency of some Green Fodders

Name of fodder	Carotene content determined by the chemical method μg. dry matter	Daily dose		Dose of standard carotene Positive control	Average growth rate per week(g)			Vitamin A equivalence of a μg. of fodder carotene
		Test material on dry basis	Amount of carotene		Test material	Standard Carotene		
	mg.	mg.	μg.	μg.	A	B	A/B	
Barley (*Hordeum vulgare*)	414·9	2·41	1·0	1·0	6·65	6·60	1·008	0·5040
Barley extract	1×10^6	..	1·0	1·0	6·43	6·52	0·986	0·4930
Elephant grass(*Pennisetum pureum*)	203·8	4·91	1·0	1·0	4·75	4·92	0·966	0·4830
Oats (*Avena sativa*)	400·0	5·00	2·0	2·0	9·70	9·00	1·078	0·5390
Kollukattai grass (*Pennisetum cenchroides*)	148·8	13·44	2·0	2·0	8·68	9·00	0·964	0·4820
Berseem (*Trifolium alexandrium*)	286·1	6·99	2·0	2·0	8·95	8·34	1·073	0·5365
Lucerne (*Medicago sativa*)	327·3	6·11	2·0	2·0	8·90	8·34	1·067	0·5335
Dub grass (*Cynodon dactylon*)	245·8	8·14	2·0	2·0	8·10	8·53	0·950	0·4750
Jowar (*Andropogon Sorghum*)	214·1	9·34	2·0	2·0	8·50	8·40	1·012	0·5060
						Average .	1·012	0·5060

a summary of the results obtained is given. It will be obvious from a perusal of the results given in the Table that the biological value of the carotene in green fodders or that in an extract of green fodder as determined by the Seshan-Sen chemical methods is almost identical with that of pure carotene standardized by spectrographic analysis. In other words, it is possible to assay the biological potency of different green fodders by the chemical method only, provided we allow the same margin of error as is inherent in the biological technique. According to Coward (1933), the biological assay of a test material with male rats over a period of 4 weeks after the depletion period may give rise to an error of +19 or —16 per cent. Consequently, if the chemical methods of analysis is to be standardized against a biological method, we cannot ordinarily expect a closer correlation than this. As the standard carotene used for reference contained about 80 per cent β-carotene and 20 per cent α-carotene (chromatographic assay), the above results appear to show that a similar proportion might exist in the carotene of the various fodder plants. It may, therefore, be assumed that the results obtained by the Seshan-Sen method of carotene estimation are a fair index of the biologically active carotene of fresh green fodder if pure carotene is used as a standard of reference and that β-carotene constitutes the major part of carotene in these plants.

(2) *Chromatographic determination of the purity apparent carotene*

A number of feeding stuffs, such as green fodders, dry roughaga, silage, etc., were studied. The carotene was extracted according to the Seshan-Sen technique, the final petroleum ether solution was passed through different adsorbents. Three different chromatographic materials were used, namely, dicalcium phosphate [Moore, 1940; subsequently used by Bolton and Common, 1942; Smith and Robb, 1943, and others], alumina (Brockmann) and Micron brand magnesia mixed with equal parts of Hyflo Super-Col. 'Heavy' magnesia of the type used recently by Austin and Shipton was not available in this country owing to war conditions. The results obtained in these experiments were then compared with those obtained by using standard carotene samples subjected to similar chromatographic analysis. The chromatography was done by first passing the solvent through the column prepared according to the usual methods, then the carotene solution was poured in before the top was dry, after which the chromatogram was developed with the same solvent. Washing was continued till the filtrate was colourless. Two types of carotene extract were used with dicalcium phosphate adsorbent, one being the petroleum ether extract before phase separation was carried out (total carotenoids), and the other being the final solution after the phase separation (apparent carotene). In both cases the solutions were dried over anhydrous sodium sulphate before passing through the adsorbent. The results obtained are briefly as follows: When the carotene from different fodder plants in petroleum ether was passed through either alumina or magnesia, there was a loss of about 14-16 per cent. An almost similar result was obtained when the standard carotene was also passed through the same adsorbents. Owing to the slowness of filtration through the finely divided adsorbents, the β-carotene part of the pigment was in contact with the adsorbent for a long period, and probably some amount of destruction took place in these cases. Chromatography through dicalcium phosphate showed negligible loss in the case of the standard carotene. The comparative results with the different materials are shown in Table II. The true carotene is expressed as a percentage of apparent carotene as determined by the phase separation method in all cases. It will be seen from the results given in Table II that a chromatographic analysis of the apparent carotene obtained by the Seshan-Sen extraction method shows it to be 85-99 per cent (average 94 per cent) true carotene in the case of green fodders as determined by Moores chromatographic technique. There is practically no difference in the results when the chromatography is carried out of the total carotenoids before phase separation or of the apparent carotene after phase separation. In the case of dry roughages, silage or cattle faeces, the ordinary chemical extraction method followed by phase separation with 90 per cent methyl alcohol does not give an idea of true carotene, and an error is caused owing to the presence of non-carotene pigments which can be removed by chromatography. In view of this fact the Seshan-Sen method of extraction of carotene has now been combined with Moore's chromatographic method for the routine analysis of carotene in all food materials (except where kryptoxanthon exists) without the necessity of phase separation with methyl alcohol. The dicalcium phosphate adsorbent has been preferred to others because it can be prepared easily and the filtration is very rapid. It is not however, suitable for separating lycopene.

TABLE II

Percentage of true carotene in apparent carotene

Source	By chromatography of apparent carotene	By chromatography of total carotenoids
Standard carotene	98·95	..
Barley plant	95·9	97·8
Oat plant	98·0	96·7
Berseem	94·2	95·9
Lucerne	98·3	94·0
Elephant grass	86·7	85·1
Dub grass	93·0	92·9
Kolluiattai grass	92·0	93·6
Spinach	97·1	99·9
Hay	..	36·5
Straw	..	0·0
Silage	..	32·5
Faeces of bullocks :		
On prolonged straw ration	..	0·0
On moderate green ration	..	78·8
On heavy green ration	..	80·0

It will be observed from the results given in Table II that the green fodders contain only a very small amount of impurity, and this must be the reason why the chemical estimation of carotene of green fodders gives a fairly close picture of its biological activity as tested against standard carotene. It will also be noticed that on heavy green fodder ingestion, the true carotene content of faeces from bullocks is as high as 80 per cent of the apparent carotene excreted. This means that it would be difficult to differentiate between the chemically estimated carotene in faeces of animals fed a heavy green ration and that assayed biologically, which has actually proved to be the case. In other words the whole of the excreted carotene appears to be biologically active because of the margin of error inherent in biological assay. On the other hand when cattle are kept on mainly dry roughage, the faeces contain much less of true carotene, as shown by chromatographic and biological assay. The observed negative balance of carotene in the case of ruminants on carotene-low rations, as described in a previous paper [Seshan and Sen, 1942 d], thus loses much of its significance. It may be mentioned that according to Russell, Taylor, Walker and Polskin [1942] no resorption of body carotene into the alimentary canal takes place in the case of hens. An alkali and alcohol digestion of feeding stuffs should, therefore, be followed by chromatographic adsorption to give a true idea of the bioactive carotene present.

(3) *Absorption of fodder carotene in avitaminotic rats*

Although the chemical, chromatographic and biological methods of assaying carotene in green fodders have given similar results, it was of interest to determine if the activity manifested in the biological assay was really due to the absorption of the total amount of carotene ingested and to what extent the amount absorbed was converted into vitamin A in the system. It has been reported recently by Ramasarma and Hakim [1942] that 10-15 per cent of carotene fed to rats at 1-2 µg. level of dosage is excreted *viâ* the faeces. It is also known that vitamin A is generally utilized better than carotene [Booher and Callison, 1939 ; Booher, Callison and Hewston, 1939 ; Guilbert, Howelt and Hart, 1940 ; Smith and Otis, 1941 ; Treichler, Kemmerer and Fraps ; 1942 ; Guggenheim, 1944]. To obtain an idea of the fate of the ingested carotene, some absorption studies were carried out.

From a sufficiently large quantity of faeces of the rats under various test-groups and the positive controls, carotene was estimated separately according to the method of Seshan and Sen, with an additional step of cooling down the final extract to 0°C, before dehydration over anhydrous sodium

sulphate. This was necessary for removing the sterols which would otherwise interfere with the estimation owing to their gradual precipitation. It was found that even without any carotene supplements there was a basal excretion of 0·22 μg. per animal per day. But this pigment was not carotene as it had not typical selective absorption in the visual region of the spectrum. The apparent excretion of carotene on various supplements at 1-2 μg. level of dosage varied from 40 to 52 per cent in the case of both the standard of reference and that supplied from the fodders. A biolgical assay of this faecal pigment showed about 25 per cent active carotene, so that the true carotene excretion was 10-13 per cent of the original ingestion. It might be mentioned here that livers of animals receiving various carotene supplements were free from reserves of vitamin A as tested by the antimony reaction. Probably the absorbed carotene from the various sources was equally utilized.

(4) *Relative efficiency of the standard carotene and preformed vitamin A*

This was studied essentially by assaying the standard carotene, which has been shown to be quite as potent as the green fodder carotene, against cod-liver oil, standardized by the spectrographic method [Coward, 1938]. $E^{1\%}_{1c.m}$ 328 mμ was taken to be 1600. The results of the bio-assays are given in Table III. It is evident from the data that the standard carotene was half as active as an equal weight of vitamin A. As the ratio of the growth rate on fodder carotene to that on standard

TABLE III

Relative efficiency of standard carotene and cod-liver oil Vitamin A at minimal levels of dosage

No of assay	Date of assay	Growth rate per week (g)	
		Vitamin A at 1 μg. level of dosage	Carotene at 2 μg. level of dosage
1	5. 5. 43 (summer)	9·33	8·98
2	4. 1. 43 (winter)	8·08	8·38
	Average	8·76	8·68

cartotene varied from 0·95 to 1·08 (average 1·01), the vitamin A equivalence of a microgram of the different fodder carotene varied from 0·48 to 0·54 μg. giving an average of 0·51 μg. (Table I). A similar relationship has been observed by other workers. It may be pointed out here that separate analyses showed no trace of vitamin A in the faeces and livers of the cod-liver oil-fed groups of rats. It might be presumed that both carotene and vitamin A were completely utilized after absorption if no change into inactive forms took place anywhere in the process of digestion and assimilation. On this basis, the efficiency of transformation of β-carotene into vitamin A can be calculated as follows. Taking into consideration the fact that the standard of reference was composed of 80 per cent β- and 20 per cent α-carotene, and assuming that 87 per cent of the carotene ingested at a level of 2 μg. was absorbed, 1 μg. of carotene appears to be as active as 0·639 μg. of cod-liver oil vitamin A. If $E^{1\%}_{1c.m}$ 328 m.μ is taken as about 1900 instead of 1600 used previously [Morton, 1942], 1 μg. of carotene would be equivalent to 0·538 μg. of vitamin A, a value which is about half the theoretical one if a symmetrical breakdown of the β-carotene molecule yielding two molecules of vitamin A occurred. It would also appear that 1 μg. of vitamin A possesses an activity of 3·1 i.μ. showing a conversion factor of i.μ./E=1631.6.

SUMMARY

1. With the object of determining the vitamin A value of carotene in different green fodders, an investigation has been undertaken to study (i) the relation between the chemically determined carotene and its biological activity as compared with that of standard carotene, (ii) the purity of apparent carotene from different sources, (iii) absorption of carotene in rats, and (iv) the relative efficiency of the standard carotene and preformed vitamin A.

2. Biological tests have shown that the chemical method of assay is a fair index of the true carotene content in green fodders, and carotene in the form of an extract is quite as effective in the system as that present in the plant tissues. β-carotene appears to be predominant in these materials.

3. Carotene in green fodders as estimated chemically is about 85-98 per cent pure as shown by chromatographic adsorption through dicalcium phosphate, whereas in other roughages a considerable part is non-carotene in nature.

4 Faecal excretion of true carotene by rats at 1-2 μg. level of dosage is shown to be 10-13 per cent according to the biological method, although the apparent excretion is as high as 40-52 per cent

5. When standard carotene is compared biologically with cod-liver oil vitamin A, the former is found to be only about half as active as an equal weight of the latter. On this basis, the vitamin A value of the various green fodders is only half of their true carotene contents.

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Waldmann Vaccine for Foot-and-Mouth Disease in Liberated Italy, 1944. R. W. Rushmore (1945). *Bull. U. S. Army Med. Dep.* **89**, 94-97.

Technique is given of preparation and dosage of a modified Waldmann's vaccine. Production is in three stages, (a) preparation of virus, (b) preparation of aluminium hydroxide solution, (c) facture of vaccine.

(a) Infective epithelium from guinea pigs is ground finely in buffer solution *p*H 7·6 and paper filtered. The filtrate is diffused under the dorsal lingual epithelium of susceptible cattle. After 24 hours the whole epithelium over the inoculated area is pelled off and placed in a freezer. When frozen solid, it is passed through a fine meat grinder, freshly distilled water is added in amount of three to one, and the mixture centrifuged at 1500 r.p.m. for 5 minutes. After drawing off the supernatant, distilled water equal to four times the original amount of epithelium is added to the sediment and the mixture is ground. This process is repeated once. The resulting solution of virus in the amount of eleven times the quantity of original epithelium is passed through Seitz Filters, first size 5 and then size 6.

(b) To prepare 20 litres of hydroxide solution, (1) 20 litres of water are warmed to 63°C. and 880 gm. of ammonium sulphate added. (2) 1500 gm. of aluminium ammonium sulphate are dissolved in 4 litres of water. (3) Solutions of steps (1) and (2) are mixed and 2 litres of cold ammonia added. (4) After 50 litres of water are added to the solution of step (3), the mixture is stirred for 15 minutes, and the supernatant fluid is drawn off by centrifuge extractor. (5) To the sediment of step (4) 60 litres water with 10 c.c. of ammonia are added, the mixture centrifuged, and the fluid drawn off. (6) This washing is repeated five times. (7) The solid portion is collected in 20 litres water— the mixture sterilized immediately at 105°C. for 30 minutes and allowed to stand for six days. Preparation of aluminium hydroxide to the point of placing it in the sterilizer must be finished within two hours' time.

(c) The following are first mixed in the order mentioned: 5 parts, aluminium hydroxide solution; 4 parts, sterile buffer solution consisting of 0·2 per cent sodium hydroxide and 0·5 per cent glycocoll solutions; 1 part, virus solution. After mixing the aluminium hydroxide and buffer solutions the container is evacuated and the virus is allowed to run in slowly and with constant agitation for 30 minutes. Formalin is added to a concentration of 0·05 per cent and the mixture agitated for further 30 minutes. The vaccine is first kept at 25°C. for 48 hours and then at 5°C. for 96 hours. A safety test is made by injecting then susceptible bovines each with 60 c.c. of this vaccine before use. The vaccine must be kept stored at 3°-7° C.

Dosage of the vaccine subcut. is 50 c.c. for adult bovines, 20-35 c.c. for calves, 5 c.c. for sheep and goats and 5-15 c.c. for swine. Revaccination every six months is recommended. [M.M. Huq.]

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ORIGINAL ARTICLES

BRUCELLOSIS IN INDIA*

By J. B. Polding, Indian Veterinary Research Institute, Mukteswar-Kumaon, U. P.

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(One text-figure)

IN INDIA, as in other countries, brucellosis usually exists within a herd in the form of a continuous infection punctuated by sporadic abortions occurring singly or in groups. Such low-grade enzootics are seen in nearly all Indian farms and many villages of the peninsula and usually produce an unending trickle of abortions of 1 to 5 per cent in the year. From time to time, however, these infections exacerbate to 5 to 15 per cent within a few months. In special circumstances major enzootics occur where the annual incidence of abortion may amount to 20 per cent during several years, whilst, when newly introduced to a susceptible herd, the disease can be severe enough on occasion to produce abortion rates of 20 to 40 per cent of pregnant cattle within a few months. Thus, brucellosis in India, while not dissimilar to the disease in other countries, probably shows greater extremes than elsewhere and attempts are made in this and the following paper to illustrate the way in which environment dominates these contrasts.

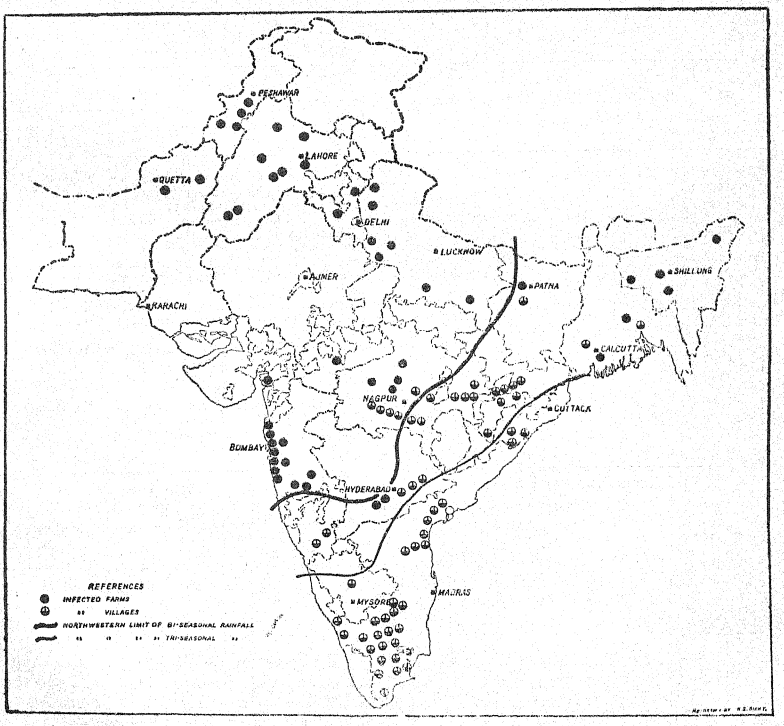

Fig. 1. Distribution of Brucellosis in India

*The data given in this paper were collected during a survey of brucellosis in India conducted under the auspices of the Indian Council of Agricultural Research, New Delhi.

Incidence and Distribution

A conspectus of the incidence of the disease in India appears in the map (Fig. 1), while Table I attempts to summarize its regional distribution in relation to climate.

Farms. Most of the organized farms in India have been visited and their stock blood-tested, while specimens from most of the others have been examined. It has been found as a result that, irrespective of situation, nearly all farms are more or less infected. Further, as most of the strains isolated from farms are of the non-indigenous species (*Br. abortus*), the origin of the infection is attributed to cattle imported from the occident. The distribution of farm brucellosis, therefore, is of small significance, being on the whole fortuitous and depending on the situations of farms and the character of their foundation stock. The rate of infection in farms, however, varies and appears to some extent to depend on climate. Thus in military establishments in areas of low rainfall, the normal annual abortion rate is 2 or 3 per cent in cows and about 1 per cent in buffaloes, but the rates in farms situated near the rain belts are sometimes higher (Table II). Again, rates in the indigenous stock of Government farms are about 1 per cent or less in the arid north-west and north and in the upper Gangetic basin, but in the wetter east they rise to 2·5 to 5 per cent or more.

Table I

Regional distribution of brucellosis in India, with reference to climate

Region	Character of region	Annual rainfall	Brucellosis in	
			Farms	Villages
Baluchistan, N. W. F. Province, Rajputana	Desert and arid hills	Uni-seasonal 5-20 in. (humidity always low)	Minor enzootics, or absent (farms infrequent)	Negligible
Punjab, United Provinces, Bihar	Cultivated plains	Uni-seasonal, 15-40 in. (humidity triseasonally low)	Moderate enzootics (farms abundant)	Do.
Bengal, Assam	Deltaic cultivation and forested hills	Bi-seasonal, 50-100 in. (humidity generally high)	Moderate enzootics (farms infrequent)	Not known
Western Hyderabad, Berar and Western Central Provinces	Cultivated plateau and forested hills	Uni-seasonal 25-50 in. (humidity triseasonally low)	Minor enzootics (farms infrequent)	Negligible
Eastern Central Provinces, eastern Hyderabad, Orissa, Madras, Mysore, southern Bombay	Richly-cultivated coastal belts, with hilly interior	Tri-seasonal, 25-50 in. (humidity always high)	Minor enzootics, or absent (farms infrequent and well situated)	Abundant
Central and northern Bombay	Cultivated coastal belt with hilly interior	Uni-seasonal, 25-100 in. (humidity always high)	Major or minor enzootics. Major epizootics (farms abundant)	Negligible

Excepting one abnormal rate of 10 per cent in both cows and buffaloes in northern Central Provinces, rates in the few farms of torrid central India are 1 per cent or less. Government farms are rare in the south and west and they seem to be but slightly affected, being mostly well situated climatically.

A fair number of *quasi* commercial private farms lie in and around Calcutta and in the coastal belt of Bombay. The humidity and rainfall here is high and abortion rates in cows and buffaloes alike may reach 20 per cent per annum, while such outbreaks seem to be getting commoner. On the other hand, private farms and *gowshalas* in the drier hinterland at present appear to be negligibly affected.

Village infection. The effect of climate is better illustrated by the distribution of the disease in villages. Obviously, in a country the size of India, it has only been possible to examine a few representative rural areas; nonetheless, discoveries here have been of far greater interest than in farms. If it is to be believed, for instance, that villages have become infected from farms, it would

be reasonable to assume that most village infection would lie adjacent thereto. A glance at the map (Fig. 1) shows that such is not the case, and indeed, village infection is commonest where farms are least numerous and *vice versâ*. Moreover, as shown elsewhere [Polding, in press] all *Brucella* strains so far isolated from these villages, differ in their typing characteristics from the *Br. abortus* strains found on farms. It seems, therefore, that village infection, occurring as it does almost exclusively on the Indian peninsula, has not been imported from Europe *via* the farms, but is an indigenous Indian infection whose distribution may prove to be of epidemiological significance. Indeed, inspection of Table I and the map (Fig. 1) reveals a curious position. Infection, as far as it has been discovered, lies mainly in the tri-seasonal rainfall area of the peninsula south-east of the thick line on the map. It has, however, advanced as far as, and follows with considerable accuracy the limits of the bi-seasonal rainfall area south-east of the thin line. Conversely, the minimum village infection seems to be in the arid north-west. It must be admitted, however, that owing to the difficulties of obtaining sufficient truly representative blood samples for testing, the final precise position in north-western villages is not yet fixed, but, so far as tests have been made, the reactor incidence is negligible.

In the south, the position is more exactly known and the incidence of agglutinin-positive village cattle runs between 10 and 50 per cent in villages where abortions occur, but is negligible where abortions are not remarked. In Table III some relevant clinical conditions are correlated with the reactor incidence in village animals of the south.

TABLE II

Above average abortion rates occurring in the wetter Military Farms

	Year	Herd strength	Abortions		
			Actual	Per cent	
Farm A					
Cows	1940	129	13	10·0	
	1941	134	16	11·9	
	1942	118	7	5·9	*Mean*=9·3
Buffaloes	1940	178	3	1·7	
	1941	232	9	3·9	
	1942	397	12	3·0	*Mean*=2·9
Farm B					
Cows	1940	137	17	12·4	
	1941	124	6	4·8	
	1942	96	19	19·8	*Mean*=12·3
Buffaloes	1940	225	6	2·7	
	1941	327	10	3·1	
	1942	432	52	12·0	*Mean*=5·9
Farm C					
Cows	1939	26	3	11·5	
	1940	30	3	10·0	
	1941	32	2	6·3	
	1942	36	4	11·1	*Mean*=9·7

TABLE III

Relation between Brucella *reactors and disease in south-Indian village cattle*

Serum reaction	Abortion	Synovial enlargements	Retention of the placenta	Sterility	Healthy	Totals
Positive	113 (66 per cent)	38 (49 per cent)	1 (10 per cent)	18 (16 per cent)	32 (10 per cent)	202
Doubtful	14	7	4	8	32	65
Negative	59	39	9	92	274	473
Totals	186	84	14	118	338	740

Susceptibility

Inextricably entangled as it is with many little-understood features of disease transmission, susceptibility *per se* is an imponderable attribute. Further, the only available data on the subject often concern as in this case, statistically unsuitable populations. Hence it becomes necessary to make the best of the available information.

Table IV

Mean incidence of abortion among various classes of stock

Class	No. of herds observed	Population observed	No. of aborters* observed	Incidence per cent
European zebu	15	1,337	273	20·4
Indian zebu, cohabiting with crosses	13	164	12	7·3
Buffaloes	15	1,880	110	5·0
Indian zebu, not cohabiting with crosses	11	4,495	167	3·7

*Aborters = animals with history of abortion at the time of a single random observation

Table V

Recurrence of abortion among various classes of stock

Class	Population observed	Abortion				Mean expectation of calf loss	
		Once	Twice	Thrice	Four times	Per aborting cows*	Per life time of 100 random stock†
European (European records)	..	68·76 per cent	..	..	..	..	..
European zebu crosses	309	71·2 per cent	25·2 per cent	3·2 per cent	0·4 per cent	1·3	26·5
Indian zebu, cohabiting with crosses	8	75·0 per cent	12 per cent	1·2 per cent	0	1·3	9·5
Buffaloes	205	92·0 per cent	8 per cent	0 per cent	0	1·08	5·4
Indian zebu, not cohabiting with crosses	134	94·0 per cent	5 per cent	1 per cent	0	1·07	3·9

*Calculated from actual recurrence of abortions in the populations observed
†Figures obtained in* multiplied by the average incidence of abortion per cent

Table VI

Pregnancy at which abortions occur in various classes of stock

Class	1	2	3	4	5	6	7	8	9	10	11	12	Population observed
European zebu, crosses	34	27	12	9	8	2	<1	<1	<1	..	..	..	228
Non-cohabiting zebu	46	26	13	9	3	2	<1	<1	<1	..	..	..	180
Buffaloes	16	39	17	10	6	6	2	<1	<1	..	..	..	130

Figures = abortion per cent occurring at the pregnancy shown

TABLE VII

Age at which foetuses are aborted

Class	Population observed	Age of foetus in months						
		3	4	5	6	7	8	9*
European (European records)	95	5	6	14	15	24	31	
European zebu, crosses	102	4	8	13	22	26	29	
Buffaloes	103	3	3	8	14	22	26	27

Figures = percentage abortions occurring at the months shown
*Buffalo's mean period of gestation = 10½ months

TABLE VIII

Abortion rate in various grades of cross-breds

Class of animal	Population	Number of aborters	Abortions per cent
Friesian (Indian records)	18*	2*	11·1
¾ grade	108*	8*	7·3
¾ ,,	329	21	6·4
⅝ ,,	152	9	5·8
½ ,,	464	14	3·0
¼ ,,	134	2	1·5
Indigenous	275	4	1·4

Mean annual abortion rate *total population* = 3·3 per cent
*Mean of three years ; the remaining figures mean of four years
(Figures by kind permission of the Director of Military Farms)

Tables IV to VIII give some abortion statistics of Indian bovines, which for purposes of comparison are grouped into : European zebu crosses, pure zebu cohabiting with European crosses, pure zebu, and buffaloes. An attempt has been made to abstract from these figures some concept of relative susceptibility ; for this purpose the mean expectation of loss of calves due to abortion, during the working lifetime of 100 random animals, has been worked out. Thus a susceptibility figure has been obtained by multiplying the mean expectation of loss of calves per aborting animal (Table V, column 7) by the mean expectation of unrepeated abortion during the lifetime of 100 random animals (Table IV). These figures are shown in Table V column 8, and, if the smallest is taken as unity, the relative susceptibility of zebu, buffaloes, cohabiting zebu, and crosses is 1·0 : 1·38 : 2·44 : 6·79, respectively. These results, together with the data of Table VIII, suggest that the greater the proportion of European blood in the animal, the greater is its susceptibility to brucellosis. At first sight, moreover, the buffalo appears to be more susceptible than the zebu. In point of fact, however, most of the buffaloes observed were cohabiting with crossbreds, so it is likely that there is little difference between the susceptibility of indigenous pure-breds.

Certain other records suggest either that the susceptibility of Indian cattle is decreasing, or that better animal management is limiting dissemination of the disease. For example, the reports of the military farms (northern circle) from 1932 to 1939 show a progressive annual decrease in the abortion rate ; a decrease which runs parallel with improvements in housing and management. On the other hand, since 1939 there have been indications that, in the busier farms at least, the rate has increased, an increase which has coincided with over-crowding and the unavoidable lessened attention due to the wartime expansion. It seems probable, therefore, that good management, rather than reduced susceptibility, was the reason for the earlier decrease. Another example of decreasing incidence is to be found in the Punjab grantee farms. In 1923 the mean abortion rate, calculated from a single random observation of five of these farms was 3 per cent of 1264 animals, while in 1939 the figures had fallen to 1 per cent of 835 animals. Here again, good husbandry is probably the cause of the diminution.

It must be noted that all the animals considered in the foregoing sketch are kept in somewhat similar conditions and that their standards of life are good, many of the pure-bred zebu herds in particular living under semi-ranch conditions in a favourable climate. But severe epidemics of abortion can occur, even in non-cohabiting zebu situated in good climates. Tables IX and X give data of one such outbreak. Ninety-five per cent of the aborted animals on this farm were *Brucella*-positive and if the annual abortion rate during the period of this epidemic be assessed at 25 per cent —a conservative estimate—the susceptibility figure of the herd works out at 11·1, *i.e.*, more than tenfold the average for this class of stock. A possible explanation of this anomaly is given in the next section.

TABLE IX

Abortion rate during an exceptional Brucella *epidemic in indigenous cows*

Year	Total breeding stock (approximate)	Number of aborters	Abortions per cent
1941	50	16	32
1942	50	9	18
Not known, believed 1942	50	16	32

Breeding herd total at the time of observation (1943) = 52
Total aborters „ „ „ = 40
„ „ per cent „ „ = 77

TABLE X

Incidence of repeated abortions during an exceptional Brucella *epidemic in indigenous cows*

	Numbers stated to have aborted			Mean expectation of calf loss	
	Once	Twice	Thrice	Per aborting cow	Per lifetime of 100 random animals
Actual	19	16	5	..	..
Per cent	49	40	12	1·65	41·25

As with cows, so with buffaloes there are exceptional rates of abortion. These abnormal rates occur in two forms, *viz.*, as severe epidemics, of which brucellosis is almost certainly the cause, and as exceptional epidemics, mostly in villages and about which little is known. The former, though perhaps getting more frequent, are not very common. The latter will be discussed in another paper.

In the absence of records, it is impossible to give figures illustrating the susceptibility of village stock, but it seems probable that they do not deviate much from the foregoing estimates for zebu cattle.

During five years of investigation not a single abortion in sheep has been attributable to brucellosis, nor can a significant incidence of reactors in these animals be recalled where sheep were aborting or where they were in contact with Brucella-infected cattle. It must be supposed, therefore, that the susceptibility of Indian sheep to brucellosis is slight.

The goat, on the other hand, seems to be rather more susceptible, for, although during this survey brucellosis has not been diagnosed in cases of clinical abortion among village goats, in two old-established goat farms, the disease has undeniably co-existed with abortions. Nevertheless, goat brucellosis in India is in no way comparable with that of the Mediterranean area.

During blood-testing work in slaughter houses, occasional isolated reactors have been encountered among goats and more rarely in sheep. It is noteworthy that these have occurred only in south

India, where brucellosis is common in village cattle. Thus, of 405 sheep and goats bled in 15 towns of south India, 11 (2·5 per cent) were positive: of 250 tested in 11 towns of north India, none was positive.

DISSEMINATION

The channels by which *Brucella* leave and re-enter their host are well known. The point to be noted is that, although these parasites are considered obligatory, in favourable circumstances they will survive outside the host and be available for dissemination. In this part of the paper an attempt is made to indicate the conditions governing this dissemination in a continent, where extreme cases give opportunities for comparison not often seen elsewhere. Throughout north-west India, there is daily scorching sunshine from April to October, natural shade is rare, and the total annual rainfall is one inch or less. In the south, conditions are the converse, temperatures vary little around blood heat, the atmosphere and land surface are moist, while natural shade is everywhere more common and for fairly long periods the sky is overcast. However, this survey has shown that brucellosis is enzootic in the south and rare in the north-west. The inference is that sunshine and dryness are inimical to *Brucella*, or conversely that equable temperatures, humidity and shade favour their survival. Certain less general observations support this premise. Earlier in this paper, the decrease of brucellosis on the Punjab grantee farms was noted. These farms lie in a semi-desert tract and for the most part their indigenous stock live and calve out-of-doors. It is possible that this is an example of self-elimination of brucellosis due to animals living in dry, sun-lit surroundings. By contrast, there are three farms in Assam, a region enduring a heavy and protracted monsoon and lying within the bi-seasonal rainbelt. The animals of these farms are similar in type and origin to those of the grantee herds, yet in 1941 the incidence of reactors amongst them was 20 per cent, whilst their abortion rate varied from 2 to 6 per cent per annum.

In another indigenous herd of the Punjab (farm I), the mean annual abortion rate during the climatically normal period 1930-1935 was 1·27 per cent of several thousand pregnancies, while during the entirely rainless period 1936-1939 the rate was only 0·66 per cent. Here, another important consideration arises, for during the rainless years the stock could not be grazed but had to be stall-fed in congested corrals. The result of this over-crowding was a severe epidemic of tuberculosis. It appears, therefore, that conditions of congestion, sufficient to produce an air-borne respiratory infection due to a relatively resistant organism, resulted in a decrease of *Brucella* infection during a period of constant sunshine and extreme dryness. Thus congestion as a factor in the spread of brucellosis may be secondary to excess of sunlight.

In contradistinction to these observations in the classical outbreak noted in Tables IX and X (farm 2). Here again atmospheric humidity was negligible and sunlight was more or less continuous. Congestion also was extreme, the animals never being out of the farm compound. The contradictory results of farms 1 and 2 may perhaps be explained on the grounds of hygienic conditions. On farm 1, aborting animals were segregated and gross infective materials, such as the placenta, were quickly destroyed. On farm 2, no such precautions were taken and general cleanliness was lacking.

In cows, therefore, it appears that, provided gross sources of contagion are removed, the sterilizing effects of sunlight are sufficient to suppress the remaining minor sources, even when congestion is great: conversely, where animals are dispersed, hygiene becomes of less importance and the effects of sunlight are paramount. Village cattle on the whole graze over wide areas and dispersion thus counteracts the lack of hygiene, but here again sunlight probably plays a major part in suppressing the disease.

There are, however, other aspects of the effect of climate on the dissemination of *Brucella*, namely humidity and rainfall, but while the possible importance of moisture is well illustrated by the restriction of indigenous brucellosis to the humid tracts of India, it is even more sharply emphasised by some aspects of the epidemiology of brucellosis in buffaloes.

Only one major endemic of brucellosis has been encountered in buffaloes (farm 3). Here the annual abortion rate was 21 per cent of pregnancies over a period of six years; this for India is certainly excessive, possibly unique. In an attempt to explain this unusual incidence, many environmental factors have been considered but the only extraordinary influence discoverable was the exceptionally heavy monsoon, with 80 in. of rainfall during the period June to September. Table XI presents a statement of the monthly abortion rates among susceptible stock, calculated from an aggregate of the returns for five years. This shows that abortion-rate for the months November to July varied little about a mean of 1·4 and although the population of susceptible animals steadily increases from January to July, there is no corresponding increase in the abortion-rate. In August, however, whilst the number of susceptible animals commences to decrease, the abortion-rate suddenly doubles and in September and October it is thrice the mean for the normal months of the year. Thereafter, the rate returns to normal. Now as the period of transmission and incubation of the disease is likely to occupy a period of 1 to 2 months, the critical period of increased infection-rate causing the increased abortion-rate during August, September and October, commences in mid-June and lasts into August. But in this period the abortion-rate, and therefore presumably the bacterial excretion-rate, is constant, whilst the corrected number of susceptible animals does not vary greatly round a mean of 1200; further the absolute number of abortions in July is the same as in May. In other words donor and receptor influences do not vary sufficiently during this period to account for the large increase of abortions in August, September and October, and this increase must be explained by a change in environment which affects bacterial survival and transmission. But in mid-June the climate changes completely from diurnal sunlight to total sunlessness and continuous rainfall and this suggests that either the absence of sunlight or the presence of moisture, or both, favours the spread of brucellosis in buffaloes.

TABLE XI

Monthly incidence of abortion among buffaloes of farm 3

Month	Term of pregnancy in months, and the abortion-liability ratio for each month					No. of abortions (5 years aggregate)	No. of susceptible animals	Ratio of column 7 to 8, per cent
	9 (3·5)	8 (3·25)	7 (2·75)	6 (1·75)	5 (1·0)			
January	12	7	11	10	10	2	124	1·6
February	7	11	10	10	46	3	154	1·9
March	11	10	10	46	131	4	314	1·2
April	10	10	46	131	134	10	567	1·7
May	10	46	131	134	116	14	909	1·5
June	46	131	134	116	80	17	1,021	1·6
July	131	134	116	80	40	14	1,377	1·1
August	134	116	80	40	21	27	1,184	2·3
September	116	80	40	21	12	36	861	4·2
October	80	40	21	12	7	21	517	4·1
November	40	21	12	7	11	5	269	1·8
December	21	12	7	11	10	1	162	0·6

Figures in columns 2 to 6 number of animals pregnant at the term shown. Aggregate for five years.

Figures in column 8=sum of the figures in columns 2 to 6, after application of the abortion-liability ratio, *plus* the figure in column 7.

As before, however, the factor of congestion complicates the picture. During the monsoon, buffaloes cannot be grazed and consequently they stand in congested cowsheds, sometimes for weeks on end. The influence of congestion may perhaps be eliminated by comparing this result with the records of farm 2 above, which, besides carrying the herd of *Brucella*-infected cattle already discussed, keeps a herd of closely cohabiting buffaloes. It is remarkable that during the epidemic no abortions occurred in buffaloes, despite their exposure to excessive infection from cows. Now on each farm the factor of congestion is probably about equal, but on farm 2, as evidenced by the spread in cows, the abundant sunlight was unable to destroy the infection, and the two environments may be compared as in Table XII.

TABLE XII

Comparison of environments in farms 2 and 3

Environment	Farm 2	Farm 3
Climate	Sunny and dry	Overcast and humid
Disposal of animals	Congested	Congested
Source of infection	Great	Ample
Result	No epizootic	Panzootic

This comparison surely suggests that in buffaloes at least rainfall plays a considerable part in the dissemination of brucellosis.

This and many other similar records lead to the same conclusion and it is tempting to accept the simple explanation that at least among buffaloes a fluid vehicle is more satisfactory for dissemination than a dry one. In this respect the buffalo's habit of wallowing requires consideration. Observations on this habit have been recorded by Minett [1947]. In north India at least buffaloes wallow voluntarily from April to October and there is a natural desire to do so when the air and water temperatures are above 85° and 77°F. respectively. The desire to wallow is intense during hot damp months, August and September in north India. During the hot dry period of April to June, water is short and the few available shrunken tanks and ponds are grossly congested, while during the rains, the animals are dispersed over a far wider expense of water.

If therefore it can be accepted that the sterilizing effect of sunlight, which is admittedly great for *Brucella*, can suppress the contagion during the sunny months, then increased infection of buffaloes during the rains, despite wider dispersion, might be partially accounted for by wallowing, especially when this is done in tanks.

Many epidemiologists have associated moisture with the spread of diseases. Rogers [1926], for example, found that cholera spreads as the absolute humidity reaches 0·4 in. and it may be noted in passing that, in lower Bengal and on the Coramandal and Malabar coasts (*i.e.*, in the *Brucella*-endemic areas of India) the absolute humidity is never below 0·4 in. About *Brucella* little has been proffered, but Cameron [1932], who has tested fairly thoroughly the viability of *Brucella*, shows on the whole that drying is harmful to the organism.

SUMMARY

The results of a survey of brucellosis in India have been analysed in an effort to trace some of the factors which control the spread of the disease in that country.

The scatter of the disease amongst farms was uniform and thus uninformative. On the other hand, an indigenous enzootic among village cattle occurred throughout the bi- and tri-seasonal rainfall areas only.

The incidence of infection in farms was often high where total rainfall was high, and *vice versâ*. The rate of village infection was negligible in semi-desert areas but considerable in humid coastal belts.

It is concluded that the sterilizing effect of continuous sunlight is paramount in checking dissemination of the disease, while humidity, rainfall and sunlessness are favourable to its spread. Nevertheless, unhygienic conditions and congestion of animals play an important part in transmission.

Abortion statistics are given for European zebu crossbreds, for pure zebus, and for buffaloes. The susceptibility of these animals and of sheep and goats is assessed.

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INDIAN SPECIES OF BRUCELLA

By J. B. Polding, Indian Veterinary Research Institute, Mukteswar-Kumaon

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NOT all *Brucella* can be exactly classified and in an examination of some 2,000 strains Huddleson [1939] refers to 13 that were not precisely *abortus, melitensis* or *suis*. Huddleson has recently intimated to me that a correspondent of his has encountered a certain atypical variant in Indonesia, which appears to be indigenous to that country. The characteristics therefore of strains isolated in India will be of interest and in this paper are described some 49 strains collected at random from this country.

Experimental

Methods

The criteria used for typing were, (1) CO_2 requirements at time of isolation, (2) H_2S secretion, (3) inhibitory action of dye media, and (4) antigenic structure. The techniques of items (1) to (3) were those of Huddleson [1939], that of (4) was based on the work of Wilson and Miles [1932]. Controls with type-specific strains were prepared throughout.

Strains were examined for dissociation as follows. Two-day-old agar cultures were suspended in 12 per cent. saline and adjusted to density 1 to 2 on Brown's scale. The suspensions were immersed in boiling water and readings for agglutination made after 5, 15, 30 and 60 minutes. The suspensions were then removed and left on the bench until the following morning when they were again examined for agglutination. A strain agglutinating in these circumstances was regarded as dissociated.

Tests for antigenic sensitivity were made as follows. Standard antigens were prepared by suspending 48-hour-old slant cultures in 12 per cent. saline and their density very accurately adjusted to 1 on Brown's scale. Stableforth's [1936] standard dried serum was reconstructed according to his directions, but the concentration of his final dilution series was halved in saline. In a series of tubes, 0·5 c.c. of the antigen to be examined was added to 0·5 c.c. of each dilution of serum in the halved Stableforth series, thus forming a titration range of 1/640, 1/800, 1/960 and 1/1120 and 1/1280 and the ultimate reaction after 24 hours' incubation at 37°C. was noted. In this range, type-specific antigen prepared in a similar way had been previously found to react to about ++ at 1/1280, and this titre was accepted as the datum with which the end points of the titrations of the field strain were compared.

Strain typing

In the intemperate climate of India, the procuring of satisfactory specimens for bacteriological examination offers many peculiar difficulties. The main troubles are the early putrefaction of dead tissues, dust contamination, long periods of transportation in warm environment and above all difficulties of communication, for abortions usually occur without notice in remote localities, which cannot be reached in time by persons qualified and equipped to take samples fit for examination. The *Brucella* strains it has been possible to secure have thus been disappointingly few, but such as they are their origin and classification are given in Table I.

In this classification, all 26 *Br. abortus* strains fell precisely into class, with no discrepancies. It is also noteworthy that all came from organized farms, most of which have carried or are still carrying European cross-bred stock, and that they occurred equally in cows and buffaloes. As regards the *melitensis* strains, in all three there was little doubt as to their classification (Table II).

It will be observed that first two strains were rough, so that their antigenic structure could not be examined, but in all probability they are normal *melitensis* types. They are alleged to have been isolated some years ago from a cow and a goat in the Punjab. The third strain was isolated recently

TABLE I

Origin and classification of Indian Brucella *strains*

Origin	*Br. abortus*			*Br. melitensis*			Unclassified		
	Host			Host			Host		
	Farm cow	Farm buff	Horse	Farm cow	Goat	Mare	Farm cow	Farm buff	Village cow
Ahmednagar	1		1						
Ambala	2	7							1
Bombay	1	2							1
Cuttack									
Ghoom	1								
Hissar					1		3		
Jarsugudda									1
Jubbulpore							1		
Kirkee		1							
Lahore	3			1					
Lucknow							1		
Meerut		1							
Mukteswar	1	1					6	2	
Mysore	1						1		
Patna									
Poona						1			
Rapur (Madras)									3
Razmak	1	1							
Sialkot		1							
Totals	11	14	1	1	1	1	12	2	6

Total: *Br. abortus*=26, *Br. melitensis*=3, unclassified=20, *Grand total*=49 strains.

TABLE II

Classification of Indian melitensis *strains*

Strain	CO_2 requirements at isolation	Excretion of H S on successive days	Growth on		Agglutination with	
			thionin	fuchsin	mono-A serum	mono-M serum
Abortus control	CO_2	+++ +++ ++ + +	—	+++	+++1/320	+1/20
Suis control	air/CO_2	+++ +++ +++ +++ +++ ++ ++ +	+++	+ —		
Melitensis control I	air/CO_2	*nil*	+++	+++	+1/20	+++1/640
Indian-*melitensis* I	air/CO_2	*nil*	+++	+++	strain rough, test not possible	
Do. II	air/CO_2	*nil*	+++	+++	do.	
Do. III	air/CO_2	*nil*	+	++	++1/320	++1/160

from a naval rating in Poona hospital, and, whilst it was able to grow in air and failed to produce H_2S, it grew but weakly on thionin and its antigenic structure appeared to be more *abortus* than *melitensis*. The reason for the appearance of these strains is obscure, but it is possible that the third at least was brought from overseas.

The twenty strains termed 'unclassified' all differed from true *Br. abortus* types in one main characteristic, *i.e.* they grew both on thionin and fuchsin. Also the majority, although undeniably *abortus* antigenically, showed a slight tendency not to differentiate so sharply as pure *abortus* types on serological tests against monospecific sera, whilst two were distinctly *melitensis* in this respect. One strain isolated from Patna and one from Mukteswar, whilst conforming to the above grouping, also grew in air at isolation.

Table III illustrates the typing results of some of these unclassified strains; those not shown in the Table were unexceptional in their behaviour and are typified by the Madras or Orissa village strains of the Table. Such strains will be referred to as "*abortus/melitensis*", or A/M for short.

TABLE III

Unclassified Indian Brucella *strains*

Origin of strain	Atmospheric requirements at isolation	H_2S Secretion	Growth on		Agglutination with	
			thionin	fuchsin	mono-A serum	mono-M serum
Lucknow (farm)	CO_2	+++ ++ ++ +	+++	+++	1/160	1/5120
Jubbulpore (farm)	CO_2	++ ++ + +	+++	+++	Negative	1/3560
Hissar (farm)	CO_2	++ ++ + +	+++	+++	1/1280	1/80
Mukteswar (farm)	CO_2	++ ++ + +	+++	+++	1/640	1/80
Do.	CO_2/air	++ ++ ++ —	+++	+++	1/320	1/80
Kanara District, Bombay (village).	CO_2	++ ++ + +	+++	+++	1/160	Negative
Orissa (village)	CO_2	+ +++ +++ +	+++	+++	1/320	1/80
Madras (village)	CO_2	+++ +++ ++ +	+++	+++	1/320	1/80
Bihar (farm)	CO_2/air	+++ +++ ++ ++ +	++	++	1/320	1/40

The + signs are purely comparative estimations of degrees of reaction. In the H_2S column the reaction was registered over five successive days.

On first examination of these strains, it was thought that a technical error had crept in, but, when the work was repeated keeping careful controls, it was found that if the above dye concentration was low enough to permit the growth of *melitensis* controls on both media, and if ordinary, European *abortus* and undoubted Indian *abortus* strains were inhibited on thionin but grew on fuchsin the results with these aberrant strains were always the same. Moreover, field strains were always typed in lots of 10 or 12 at the same time, and it is significant that out of the 49 strains no less than 20 inescapably gave this ambiguous result. It is concluded therefore that this *abortus/melitensis* type is a common indian *Brucella* variant, its constant characteristic being ability to grow on both thionin and fuchsin. Variably, it will grow in air on first isolation (two cases out of 20) ; its antigenic constitution is unstable, two strains out of 20 being *melitensis* antigenically and the rest conforming more or less to the structure of *abortus*.

Agglutination absorption tests have been made on these *abortus/melitensis* strains to observe whether they contain any additional, hitherto undescribed antigen, but this does not appear to be the case.

As to the origin of these aberrant types, it is significant that all the six strains isolated from villages of the Indian peninsula were of this nature. It is suggested, therefore, that this is a South Indian indigenous variant and that the true *Br. abortus* type, as yet exclusively found on organized farms, is an imported European strain.

Further, a consideration of the part played by climate in the dissemination of *Brucella* (Polding, in press) suggests that, if there was a spread of infection from the villages of this peninsula it would be likely to occur along the relatively humid Himalayan foot-hills and perhaps descend into the Gangetic plain. It is of interest, therefore, that the *abortus/melitensis* strains, so far isolated from organized farms, have been found at Jubbulpore—not very distant from the endemic are of Nagpur and Raipur—, and at Bihar, Lucknow, Mukteswar and Hissar, whither infection may well have spread *viâ*. the Himalaya.

Dissociation

Rough forms of *Brucella* can sometimes be recovered from the animal host, while they may also arise during prolonged artificial culture, especially if grown in fluid media. The species *melitensis* dissociates very much more readily than the other members of the genus, and among the present Indian strains two out of three *melitensis* types were rough when examined after many subcultures. Of 26 *abortus* strains, one only was partially rough in the primary culture. However, when 18 of these strains were re-tested after some 15 or 20 transfers on liver agar, six had become partially rough and two wholly rough. None of the *abortus/melitensis* strains was rough at isolation, but of 13 re-tested after some 15 or 20 transfers four had become partially rough and one wholly rough.

Antigenic sensitivity

Most of the present collection of Indian strains have been tested for differences in antigenic sensitivity but within the limits of ordinary error no differentiation has been observed, — even the *abortus/melitensis* forms being unexceptional in this respect. Some of the results of this work are shown in Table IV.

Virulence

As white mice are required in large numbers in assays of virulence, and as these animals are not easy to obtain in such numbers in India, only a few Indian *Brucella* strains could be examined for this property. The test is described by Priestley and McEwen [1938] and the results of some of the present estimations are shown in Table V. For comparison, tests on three foreign vaccine strains are included in this Table. Again, for control purposes it was necessary to compare the virulence of Indian strains with that of type-specific strains from abroad, and in so doing few mice were left for testing the local strains ; even then, the interest centring round the *abortus/melitensis* type led to four of these and only one *abortus* and one *melitensis* being examined.

TABLE IV

Antigenic sensitivity of Indian Brucella *strains*

Strain	Type	Titre
F 39	A/M	+1/1120
F40	A/M	++1/1280
F41A	A/M	+1/1280
F41B	A/M	+1/1120
F42	A/M	+1/1280
F43	A .	+1/1120
F44	A/M	++1/1120
F45	A .	++1/1120
F46	A/M	+++1/1120
F47	A/M	++1/1120
F48	A/M	++1/1280
Control (European)	A .	++1/1280

A=*Br. abortus* A/M=*Br. abortus/melitensis*

TABLE V

Virulence of Indian field strains

Strain	Bacterial doses					
	16×10^9	8×10^9	4×10^9	2×10^9	1×10^9	$0{\cdot}5\times10^9$
F.46 Indian CO_2 A/M			4	3	2	3
F.45 Indian CO_2 A			4	2	0	1
F.41 Indian CO_2 A/M			5	5	2	2
F.40 Indian CO_2 A/M			5	3	0	1
F.36 Indian CO_2 A/M			5	4	4	1
F.34 Indian M			5	5	4	4
A.1 European A			3	1	0	1
M.1 Malta M			5	3	1	1
V.1 Cotton's 19	5	4	1			
V. 4 McEwen's 45(6)	2	1	1			
V.5 McEwen's 45(20)	2	5	2			

Numbers=dead mice out of 5.
A=*Br. abortus* M=*Br. melitensis* A/M=*Br. abortus/melitensis* CO_2=CO_2 sensitive

The Indian strains tested were recently isolated and, except the *melitensis* strain, were CO_2 sensitive. As might be expected, the order of virulence proved to be: *melitensis* most virulent *abortus-melitensis* of intermediate virulence, and *abortus* least virulent. As repeated sub-culturing is supposed to lead to loss of virulence, a comparison with the overseas type-specific strains must be made with caution. It is to be remembered that the Maltese strain was isolated six years previously and the English *abortus* strain must be many years old. After making due allowance for this, however, the experiment suggests little difference between Indian and overseas species. To check up this suggestion, in Table VI a comparison is shown between the Indian strains and an average result of four recently isolated English *Br. abortus* strains typed by Priestley and McEwen [1938]. If it can be believed that tests done by different persons are comparable, the foregoing conception of virulence seems to be substantiated.

DISCUSSION

The apparent rarity of *melitensis* strains in India is note-worthy, for while corroborating the belief of most contemporary observers, it accords ill with the early history of human infection, which for this reason merits a brief scrutiny. From the year of Wright and Smith's [1897] demonstration

of the agglutination test for brucellosis until the far-reaching revelations of the Mediterranean Fever Commission [1905-07] an ever-growing number of reports were published of undulant fever in India.

TABLE VI

Comparison of the virulence of Indian and European strains

Strains	Bacterial dose			
	4×10^9	2×10^9	1×10^9	$0{\cdot}5\times10^9$
Indian M	5	5	4	4
Indian CO_2 A/M*	5	4	2	1
Indian CO_2 A	4	2	0	1
McEwen's CO_2A†	4	2	1	0

Numbers=dead mice out of 5

* Average of 3 Indian village A/M strains

† Average of 4 of McEwen's *abortus* strains (CO_2-sensitive and virulent), Priestley and McEwen [1938]

A=*Br. abortus* M=*Br. melitensis* A/M=Br. *abortus/melitensis* CO_2=CO_2—sensitive.

It is indeed remarkable that with the crude facilities and techniques of those times, investigators were apparently able to isolate what seemed to be the causal organism with ease and certainty, *e.g.* from 11 humans in Multan and Ferozepore [Lamb and Pai, 1906], and from goats also in Ferozepore [Froster, 1906]. Concurrently, numerous serum-positive cases of typical fever were described from all over India and a perusal of the contemporary journals forces the conclusion that undulant fever was enzootic in certain military cantonments, with the inference that the Punjab at least was widely infected. What is more, a scrutiny of the records cannot fail to convince the critical that a gram-negative, CO_2-insensitive, cocco-bacillus, agglutinable by *Brucella*-specific serum, was being isolated. This, together with the fact that CO_2 was not required at isolation, is an indication that the species concerned was *melitensis*. On the other hand, agglutination reactions were generally weak, and demonstration of infection in animals was infrequent and confined to a few cantonment goats only.

In 1907, the spate of case reports suddenly subsided and at the present day it is difficult to obtain more than very rare reports of single scattered cases of undulant fever. But this surprising state of affairs can only be explained by one of three propositions, (1) that the earlier reports were incorrect, (2) that in the period 1907-1939 human brucellosis in India had practically died out, or (3) that undulant fever still occurs frequently but is either not reported or not diagnosed.

The earlier work must either be disbelieved as a whole, thus discrediting the work of several specialists, or accepted as a whole. On the other hand, it is past belief that at the present time frequent cases of so obvious a disease as *melitensis* infection could be missed in modern army hospitals. In the face of this, an explanation must perhaps be sought in the admittedly rather improbable proposition 2.

Now it is odd, to say the least of it, that case reports in India subsided so soon after 1907, the very year succeeding that in which the infection rate in the British garrison at Malta was so greatly and dramatically diminished. But nearly all the cases of genuine Malta fever reported in India, occurred in cantonments, and in the words of Scott [1939], 'The disease was shown to be widespread in north-west India and *especially in places where British troops were stationed*' (The italics are mine). Can it be accepted then, that infection in indian cantonments was brought by trooping movements from Malta, that dissemination was entirely localized, and, to round off the premise, that when fresh sources of infection from Malta failed, in the absence of a sufficient animal reservoir and possibly in the face of stricter boiling of milk, the local infection partially or wholly died out ?

During the present investigation constant attempts have been made to stimulate medical interest in brucellosis ; but it has been rightly contended that during the wartime emergency little effort could be spared for a disease of such *apparent minor importance*. The securing of human strains has, therefore, been rendered almost impossible by the unavoidable circumstance of war.

Nevertheless, it is of deep interest why a disease, so prevalent in man on the goat-populated Mediterranean littoral, should be apparently so rare in man in India, where in certain parts at least sheep and goats are abundant. At one time the simple explanation of the almost universal Indian custom of the boiling of milk was stressed as the explanation, but in France Taylor, Lisbonne, Vidal and Hazemann [1938] have clearly shown that at least among breeders of goats and sheep, and possibly also amongst all who come in close contact with these animals—such as small-holders, house-wives and butchers—some 40-60 per cent of infection is transmitted to man by contact. For a considerable section of the Indian public, therefore, the 'boiled milk' explanation fails and, if it can be assumed that *melitensis* fever in man would be diagnosed and reported were it a sufficiently prevalent disease in this country,—an assumption which is probably valid—then it appears that the reason for its rareness in man must be its rareness in animals—and as will be shown elsewhere (Polding, unpublished), this appears to be the case as far as sheep and goats are concerned.

Br. melitensis is not, however, the sole *Brucella* species pathogenic to man, and it seems possible that a variable number of Indians must become infected with *Br. abortus* from contact with animals, in which there is no room for doubt that *Br. abortus* infection occurs. Rare serum-positive patients, suffering from fever, are reported from time to time, notably in the North-West Frontier Province and Punjab, but until the responsible strains are isolated and typed, which has not yet been done, it will not be known whether these fevers are *melitensis* or *abortus* in type, although the latter seems more likely.

On the face of it, therefore, it seems that, if *melitensis* infection has occurred at one time in India, it is not very prevalent today. The *abortus* type is known to occur in cattle and is likely to occur to some extent in man. Sheep and goats appear to be relatively free from either infection, whilst there is no reason to suppose that the *suis* species occurs at all.

The existence of the aberrant *abortus/melitensis* strain holds much interest. Huddleson's correspondent, cited earlier, observed similar characteristics in his aberrant Indonesian strains. In India, the only truly enzootic area, where the incidence of brucellosis in native village cattle is significant, is the south-eastern seaboard. This is also the area of constantly high atmospheric humidity, equable temperature, and a tri-seasonal rainfall. This portion of India is, therefore, not only nearest to Indonesia geographically but also climatologically. Consequently, it is tempting to suppose that this is a middle-asiatic variant, which is adapted to survival in regions favourable to the cultivation of rice.

SUMMARY

Forty-nine Indian field strains of *Brucella* were typed and their dissociability, agglutinability and virulence assessed. They were unexceptional on all the last three counts. Of the 49, 26 were pure *abortus*, 20 were intermediate between *abortus* and *melitensis*, 3 were *melitensis* and there were no *suis*. All six strains isolated from village cattle of the *Brucella* enzootic area of the peninsula were of the intermediate type and it is suggested that this is an indigenous Indo-Indonesian variant.

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EXPERIMENTS ON THE TRANSMISSION OF SURRA

BY THE TICK *ORNITHODOROS THOLOZANI* LABOULBENE AND MEGNIN

By S. K. Sen, Imperial Veterinary Research Institute, Mukteswar

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In a paper published in 1922,* Cross and Patel, in the Punjab, brought forward some evidence to show that, under experimental conditions, surra was capable of being transmitted through the agency of *Ornithodoros tholozani* Laboulbene and Megnin†, a tick of fairly common occurrence in that province. They further observed that these ticks transmitted the disease to healthy rabbits 67, 83 and 101 days after feeding on a surra-infected animal, but that they proved non-infective when tested one minute to 46 days after their infective feed. In fact that the ticks required at least an interval of 46 days to become infective for healthy animals led them to postulate the occurrence of a cyclical development of the trypanosome within this invertebrate host, on the analogy of what is known to occur in certain other forms of protozoan infection. In a later communication, however, Cross and Abdulla Khan [1923] stated that 'these ticks were found capable of spreading the disease to a healthy animal 17 days and one month after feeding on an infected animal', whilst Kahan Singh [1925] fixed the maximum limit at the 212th day. It is noteworthy that this last worker also reported having obtained positive results when the ticks were fed by the 'interrupted method', and furthermore, these results appeared to him to point to the conclusion that 'not less than 10 ticks of this species are capable of transmitting the disease.'

On the other hand, a series of experiments carried out by Yorke and Macfie [1924], by means of infected *O. tholozani* tick forwarded to Liverpool by Cross from India, yielded results of an entirely negative order. It occurred to these workers that a possible explanation for the failure of these ticks to infect was the fact that, immediately after the infecting feed, they were subjected to relatively low temperature during transit from India to England in the month of December. In order to examine this question, they obtained a second supply of ticks that had been fed on a heavily infected dog on 23 July, 1923. These ticks, which were received by them on 24 August, were allowed to feed on a rabbit at frequent intervals, but no infection resulted. In commenting on these results, the authors remark: 'We have no explanation for these negative results, but the subject is one of such practical importance as to demand re-investigation.'

The experiments described in this paper were undertaken with a view to further pursuing the question and more particularly with the object of obtaining any indication as to the occurrence of a cyclical development of the surra trypanosome in *O. tholozani*, a supply of these ticks having been obtained through the courtesy of the Principal of the Punjab Veterinary College, Lahore.

For providing the infective feeds, guinea-pigs were used showing trypanosomes at the rate of 12 to 40 per field. A total of 502 ticks were thus infected and they were later placed in seven groups, as shown below, in accordance with the date on which they were fed and the temperature at which they were maintained.

Group I. 26 August 22 ticks fed on a guinea-pig showing trypanosomes at the rate of 12 per field.
Ticks were kept at room temperature (about 20°C.).

Group II. 27 August 56 ticks fed on a guinea-pig showing trypanosomes at the rate of 15 to 20 per field.
Ticks were kept at room temperature (about 20°C.).

Group III. 27 August 56 ticks fed as above, but kept in incubator at 25°C.

Group IV. 1 September About 49 ticks fed on a guinea-pig showing trypanosomes at the rate of 20 per field.
Ticks were kept at room temperature (about 20° C.).

Group V. 1 September About 48 ticks fed as above, but kept in incubator at 25°C.

Group VI. 2 September About 137 ticks fed on a guinea-pig showing trypanosomes at the rate of 40 per field,
Ticks were kept at room temperature (about 20°C.).

* Paper read at the Jubilee Session of the Indian Science Congress held in Calcutta, January 1938. Revised and re-written.

† This tick has also been known by the names of *O. crossi* Brumpt and *O. papillipes* Birula, whilst, in a recent paper (*Ann. Parasit. Hum. Com.* 1946, 21, 74-88), Desportes and Campana have designated it *O. tholozani* V. *crossi*.

Group VII. 2 September About 135 ticks fed as above, but kept in incubator at 25° C.

The object of keeping a proportion of the ticks in the incubator was to determine the effect, if any, of a relatively high temperature on the viability of the trypanosomes after being ingested by these ticks, this possibility, as already mentioned, having been suggested by Yorke and Macfie. The infectivity of ticks taken from each of these seven groups was later tested at various intervals on healthy rabbits, a note being taken of the actual number of ticks fed on each occasion in order that, in the event of the result being positive, some indication might be obtained as to the minimum number of ticks necessary to produce infection. The experimental rabbits were observed for about three weeks and the results of these experiments are summarized in Table I.

TABLE I.

Result of experiments with ticks to produce infection

Group No.	Number of ticks fed	Interval between infective and 'healthy' feed	Result
VI	30	48 hours	Negative
VII	30	48 ,,	,,
IV	30	7 days	,,
V	30	7 ,,	,,
I	5	20 ,,	,,
II	30	21 ,,	,,
III	30	21 ,,	,,
I	5	30 ,,	,,
VI	33	34 ,,	,,
VII	35	34 ,,	,,
I	5	40 ,,	,,
VI	32	48 ,,	,,
VII	33	48 ,,	,,
I	5	50 ,,	,,
Total	333		

It will be seen from the foregoing Table that a total of 14 trials, involving the use of 333 ticks, were carried out and the interval between the infective and the 'healthy' feed ranged from 48 hours to 50 days, this range, in view of the observations recorded by Cross and Abdulla Khan [1923], being considered sufficiently long and varied to allow for the reproduction of the disease in experimental animals. The results, however, were of an entirely negative order and were, therefore, in accord with those recorded by Yorke and Macfie.

SUMMARY

The tick, *Ornithodoros tholozani*, was incapable of transmitting surra, under experimental conditions, from infected guinea-pigs to healthy rabbits.

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TRANSMISSION OF RANIKHET DISEASE OF FOWLS

By J. A. IDNANI and C. SEETHARAMAN, Indian Veterinary Research Institute, Mukteswar-Kumaon

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ALTHOUGH an efficient prophylactic vaccine has now been evolved for use in the field, much can be done apart from this to control the disease in unvaccinated areas by means that can easily be employed by poultry farmers. The experiments described in this paper were designed to examine some aspects of this control.

Various views and experimental data on the dissemination of Ranikhet disease under natural or artificial conditions are recorded in the literature on the subject. Outbreaks of the disease occur mostly on the introduction into healthy flocks of newly purchased birds which are either diseased or are in the incubation period or have just recovered, and the dissemination of virus is brought about by infected exudates and excreta. Outbreaks in flocks with no history of contact infection are attributed to the dissemination of the virus by such mechanical carriers as crows, pigeons, vultures and other wild birds, but there is as yet no proof of this nor has the virus been isolated from any such carriers. The possibility of its transmission by fowl ticks (*Argas persicus*) has been investigated by Komarov [1940] with negative results. We have observed that lice from an infected bird on being transferred to a healthy one did not transmit the disease. Thus the digestive and respiratory tracts appear to be the usual routes of infection.

Extensive studies have been made on the bionomics of the causal agent in countries where the disease occurs. Virus suspension, on which most of the experimental work has been done, at ice temperature retains its viability for several months and this explains the wide incidence of the disease during the cold season. However, the virus is very fragile and is readily affected by a warm atmospheric temperature and by sunlight. At 50°C. to 55°C. the virus is rendered inert by 30 minutes' exposure and killed by direct sunlight in one hour. In garden soil or in stagnant water at room temperature (temperature not given) it became non-infective in four days [Farinas, 1930]. In contaminated brooders Dobson [1939] was able to recover the virus for a period of seven weeks. In the light of these data a vigilant breeder can achieve considerable success in mitigating losses. The secretions, excretions and tissues of affected fowls are all infective, providing wide scope for dissemination. Doyle [1927] demonstrated that the disease is readily produced by a light application over the mouth and pharynx with a swab of cotton wool soaked with mouth exudate of an affected bird. Even small quantities of the virus set up the disease by intravenous, subcutaneous and intraperitoneal inoculations, as well as by scarification and instillation into the eye or into the respiratory tract [Stover, 1942] of susceptible birds. Under natural conditions, apart from direct contact— a term which is somewhat meaningless—ingestion of infected material is reported to be the most common method of transmitting the disease [Farinas, 1930].

EXPERIMENTS ON INGESTION

Virus in pills or capsules. Fresh spleens from two fowls, which died of Ranikhet disease were made into a paste and weighed into 0·5 gm. quantities. Such weighed quantity was made into a dough with wheat flour and formed into pills with particular care that the surface of the pill should not be contaminated. Similar quantities of tissue were enclosed in gelatin capsules. Four birds were given pills and four gelatin capsules, each receiving one pill or one capsule, which passed through the gullet into the crop. The birds were then kept isolated from one another for 8-10 days but no reaction ensued and all were later proved to be susceptible. The experiment was repeated on four fowls, 0·5 gm. infected spleen in dough pills being given to each bird. They too reacted to inoculation of virulent virus fifteen days later.

Virus mixed with grain. Five fowls were given with their grain ration pieces of fresh infected spleen. The feeding was individual and each fowl got 0·5 gm. spleen tissue after which some more uncontaminated grain was given. The minced spleen was well mixed with grain (crushed maize, crushed barley, bran) and was not wetted with water before it was fed. The birds remained unaffected ; eight days later they reacted to test virus and died of Ranikhet disease.

Virus in water. One hundred cubic centimetres water into which 2·0 gm. fresh infected spleen had been stirred was put into the drinking pots of each of the seven birds. Each bird took approximately 17 c.c. of the suspension within half an hour and was then segregated Another fowl was given 0·5 gm. of tissue suspended in 5 c.c. water, the mixture being pipetted on to the back of the throat. All the eight birds contracted the disease and died within 4-7 days after the infective drink.

Experiments on inhalation

Two wooden boxes were so constructed that through an opening on one side the neck of the fowl would protrude, the base of the neck being held in position by means of a cardboard collar. The two boxes were so placed that there was a distance of two feet between the beaks of the two birds, one infected 48 hours previously with Ranikhet disease virus and the other healthy. The entire structure was covered with a piece of muslin cloth for ventilation. After three hours' exposure the healthy bird was removed; four days later, it developed symptoms of the disease and died on the fifth day. The day after removal of the first healthy bird, another healthy one was exposed under identical conditions to the infected bird now in its third day from inoculation. In this case also the disease was conveyed through the expired air to the healthy bird which died on the seventh day. Another healthy bird was similarly exposed to a fowl infected four days previously showing signs of acute illness. In this case infection was not transmitted and the bird was later found susceptible. In three subsequent trials the distance was increased to three feet, the time of exposure and other conditions remaining unchanged. In all these, however, the results were negative.

In a second series of experiments carried out under similar conditions except that the muslin was replaced by thick cloth of close texture so as to minimise air currents. a healthy bird was exposed for three hours to an infected fowl at a distance of two feet. Fifteen days later the exposed bird proved susceptible to Ranikhet virus. This experiment was repeated thrice with similar results. In another experiment a healthy bird was exposed under the same conditions but for a duration of six hours with negative result. In a third experiment of this second series involving three infected and three healthy fowls the distance was reduced to one foot. However, in only one case did the healthy bird contract the disease, dying on the 7th day.

Discussion

It appears that Ranikhet disease virus is rendered inert in the digestive tract. The above results may be explained by the fact that, whereas solid food is quickly swallowed, during the process of drinking fowls fill the mouth, raise the head and swallow each gulp gradually so that the mouth, pharynx and larynx become contaminated. It seems likely therefore that infection takes place via. the nasopharynx and before the virus enters the crop, since swabbing the throat with the virus easily produces infection. There is little doubt however, that under natural conditions droplet infection is the most common method of transmission. The experience of Farinas [1930] that infection is not readily transmitted between cages may be explained by the lack of air currents favourable for spread. In droplet infection the chances of transmission by coughing or breathing largely depend on the distance between the infected and susceptible. If the distance is too great, the virus may be diluted beyond its infective potentiality. After expulsion the virus may be carried by air currents some distance from its source and contaminate the surrounding areas. This explains how, as sometimes noted in practice, an outbreak of Ranikhet disease starting at one end of a poultry farm may spread in a few days to the interior and even to the farthest corner of the farm.

Summary

1. Ranikhet disease virus, enclosed in a gelatin capsule, within wheat dough or mixed with grains, when introduced directly into the digestive tract does not produce the disease in susceptible birds.

2. Susceptible birds contract the disease when allowed to drink water containing Ranikhet disease virus in the form of tissue suspension from infected birds.

3. A susceptible bird contracts the disease when exposed for three hours or more to an infected one at a distance of not more than two feet under conditions suitable for the play of air currents.

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POSSIBILITY OF COLOURING *VANASPATI* WITH RATANJOT TO PREVENT ITS USE AS AN ADULTERANT IN *GHEE*

By Noshir N. Dastur, A. Kannan and Zal R. Kothavalla, Imperial Dairy Research Institute, Bangalore

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Hydrogenated vegetable oils, or what are popularly known as *vanaspati*, are used now a days in large quantities as cooking media. They provide a cheap substitute for *ghee* for this purpose and for this reason are normally made to resemble *ghee*, as closely as possible, in appearance. The two being visually very similar there is always the temptation to vendors to mix the cheaper product (*vanaspati*) with *ghee* and to pass the mixture off as genuine *ghee*. The detection of the admixture of these two is further made difficult by the fact that there is great variation in the chemical composition of *ghee* and it is not always possible to distinguish between genuine *ghee* and adulterated products, even by routine chemical methods.

The ordinary purchaser must rely on his sight to distinguish between the two products and in order to enable him to do this it has been strongly urged that all *vanaspati* manufactured in India should be given such a distinct colour that it may not be mistaken for *ghee* even by a casual purchaser. Deep yellow (turmeric) and orange colour (like the one given by annatto) are obviously not suitable. Next to these two, red is the most agreeable colour. It was therefore, decided to carry out trials to determine the most suitable variation in red to colour *vanaspati* and 'rosé red' or the tint commonly known as '*Gulabi*' was finally decided upon. The selection of this tint no doubt was rather arbitrary, considering the fact that the accepted colour of natural fats is yellow but that made the selection of a tint other than yellow all the more necessary, as long as it was not repulsive to the consumer and fulfilled all the requirements of a cooking medium; for, after all *vanaspati* is used for this purpose only.

The dye to be used for colouring edible products like *vanaspati* must fulfil certain essential requirements such as, (*a*) it must be easily available, (*b*) it should be non-toxic in raw state or even after heating to the frying temperature of *vanaspati*, (*c*) it should not in any way affect the quality of *vanaspati* by lowering its keeping quality or imparting a foreign flavour to it and (*d*) the coloured *vanaspati* must be stable to light and heat to a reasonable extent so that the colour may not be destroyed during storage. Preliminary examination of several natural and synthetic dyes showed that the red dye obtained from the *ratanjot* roots, *Onosma echides*, fulfilled all the above conditions. *Ratanjot* plants grow in abundance in colder regions of India. The roots form the main source of the dye.

Experimental

Isolation of the Dye. The colouring principles of *ratanjot* are insoluble in water but freely soluble in fats and common organic solvents. The dye was usually concentrated by extraction with ether. Before extracting the roots with ether, they were steeped in ten parts of water for about 24 hours. The roots were dried at room temperature and fibres and other impurities were removed. The loss in weight during the steeping was of the order of 6 per cent. The percentage of impurities, including fibres, etc., varied from 13 to 18 per cent. Thus on the whole the quantity of extractable roots was about 80 per cent of the original roots taken. Steeping was also done in 5 per cent salt solution. This however, did not give any better results. The concentration obtained was to the extent of 6 per cent calculated on the basis of the weight of cleaned roots. The constitution of the colouring principles of the *ratanjot* roots has not been studied in detail but presumably it is closely related to that of the alkanet roots, *Anchusa tinctoria*, lam. [Perkin and Everest, 1918.] Nargund [1942] suggested the formula $C_{30}H_{28}O_8$ for the colouring matter. It melts at 220°C., with previous softening at about 190°C.

Ratanjot roots are commonly sold in the bazar. The roots on storing do not seem to loose their colour imparting property. The colour concentrate, extracted with ether, when stored at 37°C.,

for eight months did not suffer deterioration in the colour giving property and can thus be taken as fairly stable under ordinary storage conditions. On the other hand, if the dye is heated to a very high temperature it does not give any red colour to fats but will still give the characteristic blue colour with alkali which is now taken as a specific test for indicating the presence of *ratanjot*.

Colouring vanaspati. The concentrate obtained by extraction with other is not directly used for colouring *vanaspati* as it is found that all of it is not soluble in fats. One hundred grams of *vanaspati* require nearly 0·25 gm. of this concentrate to give an almost saturated solution of colour in fat. To extract all the colour, the mixture is repeatedly heated to about 60°C. with constant stirring. This is then filtered and the deep red *vanaspati* used for colouring any further quantities of *vanaspati* to the required intensity is obtained. On an average *vanaspati* of the kind ordinarily offered in the market requires 5·5 gm. of the concentrated coloured *vanaspati* of the type, described above, to colour 1000 gm. of it to one red unit, as measured in Lovibond Tintometer using 1 cm. cell, the colour comparison being carried out at 50°C. This gives a concentration of 0·0096 per cent. Under practical trade conditions a colour intensity of seven red units is suggested as most suitable. A high intensity of colour can easily be given, but for the purpose in view this is not necessary, as the presence of *vanaspati* so coloured can fairly easily be detected as has been described later.

Stability of coloured vanaspati *to light*. Investigations were carried out with different brands of *vanaspaties* available in the market, and they were coloured to three different intensities, viz. five, six and seven red units. For the sake of brevity, however, results obtained with only one popular brand of *vanaspati*, made from the groundnut oil, and coloured to seven red units, have been dealt with below.

The *vanaspati* in question was kept in glass bottles stoppered with corks and exposed to ordinary daylight in the laboratory. The changes in colour, peroxide values and acidity were recorded at intervals. Peroxide values were determined by the method described by Dastur and Lea [1940] and expressed as ml. of N/500 thiosulphate per gm. of fat. Acidity was estimated by the method described in the Handbook of the A.O.A.C. [1945] and expressed as percentage of oleic acid. The results are given in Table I.

TABLE I

Effect of diffused sunlight on coloured vanaspati

Time interval in days	Description of the samples	Colour reading			Decrease in red colour (per cent)	Acid value (percentage of oleic acid)	Peroxide value
		Red	Yellow	Neutral			
0	*Vanaspati* (A)	0·3	1·0	0·2	..	0·06	0·47
	coloured (B)	7·1	1·3	0·2	..	0·08	0·47
7	A	0·2	1·0	0·1			
	B	6·9	1·4	0·1	2·8		
14	A	0·2	1·0	0·1			
	B	6·9	1·3	0·1	2·8		
28	A	0·2	0·6	0·2			
	B	6·9	1·5	0·1	2·8		
42	A	0·2	0·4	0·1			
	B	6·7	1·3	0·1	5·6		
56	A	0·2	0·5	0·2			
	B	6·5	1·5	0·1	8·4		
70	A	0·2	0·8	0·2			
	B	6·5	1·6	0·1	8·4		
86	A	0·2	0·7	0·2			
	B	5·9	1·7	0·1	16·9		
100	A	0·1	0·5	0·1		0·06	
	B	5·7	1·7	0·1	19·7	0·08	
132	A	0·0	0·4	0·1			
	B	4·8	1·6	0·1	32·4		
157	A	0·0	0·3	0·1			25·78
	B	4·4	1·5	0·1	38·0		14·89

The main conclusions which can be drawn from the data given in Table I are as follows:

(*a*) The colour of *ratanjot* is somewhat sensitive to light. It is, therefore, necessary to see that coloured *vanaspati* is not exposed to light as far as possible. This will not be a difficult matter under trade conditions as the *vanaspati* is invariably sold packed in tins.

(*b*) The presence of *ratanjot* does not in any way affect the keeping quality of *vanaspati*. The acidity does not show an increase even after an interval of 157 days.

(*c*) The peroxide values indicate that *ratanjot* seems to have anti-oxident properties.

Stability of coloured vanaspati *to heat. Vanaspati* samples were kept in glass bottles and stored at:

(*a*) Room temperature in dark.

(*b*) At 37°C. in an incubator.

(*c*) At 42°C. in an incubator.

The results obtained are shown in Table II.

TABLE II

Effect of storing coloured vanaspati *at different temperatures*

Time interval in days	Room temperature	Percentage of decrease in red colour at:	
		37°C.	42°C.
0	..	..	..
35	0·0	0·0	7·0
62	0·0	0·0	11·2
100	0·0	4·2	16·9
130	2·8	8·4	28·1
215	1·4	12·6	36·7

The above date indicate that:

(*a*) With the increase in temperature of storage, a decrease in colour intensity is observed. Considering the extent of the decrease noted, this may not be considered serious under practical trade conditions, as *vanaspati* may ordinarily be expected to be disposed off in 3-4 months' time after its manufacture.

(*b*) Under ordinary climatic conditions, a decrease of about 10 per cent in the colour intensity may be expected in about six months' time. This was further borne out by the fact that coloured *vanaspati* samples kept in sealed tins under climatic conditions prevailing in Bangalore and Bombay during summer months showed a decrease in colour to the extent of 11 per cent after six months' storage in Bombay and 1 per cent after three months' storage in Bangalore.

(*c*) During these studies a record of the development of peroxide value and acidity was also kept. No difference in the keeping quality of the coloured *vanaspati* and original samples was observed.

Effect of adsorbents on coloured vanaspati The edible colours as a rule are removable to varying degrees by the use of different kinds of adsorbents. This fact is in practice is utilized for bleaching vegetable oils used in the manufacture of *vanaspati* itself. It is, therefore, to be expected that these adsorbents would also remove the colour from coloured *vanaspati*. To study the effects of the various adsorbents quantitatively, therefore the following experiments were carried out:

(*a*) One hundred grams of coloured *vanaspati* were mixed with known amounts of rice husk, charcoal, Fuller's earth and kaolin. The mixtures were heated off and on for three hours at 70°C. with occasional stirring. The fat was then filtered and the colour compared. The results obtained are given in Table III.

TABLE III

Effect of different adsorbents on coloured vanaspati

Amount of adsorbents used (per cent)	Rice husk	charcoal	Fuller's earth		Kaolin	
	Colour after treatment (red units)	Percentage decrease	Colour after treatment	Percentage decrease	Colour after treatment	Percentage decrease
0	7·0	..	7·0	..	7·0	..
1	4·7	32·9	6·3	10·0	6·2	11·4
2	3·5	50·0	5·3	21·4	6·2	11·4
3	2·5	64·3	4·6	34·3	6·0	14·3
4	2·1	70·0	4·4	37·1	5·9	15·7
5	1·8	74·3	3·8	45·7	5·8	17·1
6	1·6	77·1	3·5	50·0	5·7	18·6
7	1·3	81·4	3·1	55·7	5·5	21·4
8	1·1	84·3	3·1	55·7	5·5	21·4
12	0·9	87·1	2·9	58·6	5·2	25·7

The *ratanjot* colour is removed fairly easily by different adsorbents. Activated charcoal seems the cheapest and best adsorbent. Based on this property, a method has been suggested later for detecting the presence of *ratanjot* even in a very small quantity. This removal of colour from coloured *vanaspati* by adsorbents, it may be argued, is likely to provide a loop-hole to the trade, viz., to first refine the *vanaspati* before mixing it with *ghee*. It is however, felt that the separation of adsorbents from the fat may not prove such an easy matter for small merchants. The treatment cannot be adopted on such an extensive scale as theoretial results would suggest and yet go undetected.

Based on the fact that the colour of *vanaspati* can be removed by adsorbents, some workers have suggested that addition of sesame oil to *vanaspati* would provide a more effective way of detecting the presence of *vanaspati* if used as an adulterant with *ghee*, viz., the application of a specific test 'Furfurol test,' for sesame oil. Experiments were, therefore, carried out using coloured *vanaspati* containing 5 and 10 per cent sesame oil. These samples were treated with 5 per cent charcoal and Fuller's earth. The decrease in the colour intensity of the Furfurol reaction and the colour of *vanaspati* was observed as noted in Table IV.

TABLE IV

Percentage of decrease in the intensity of colour when treated with adsorbents

Amount of sesame oil in coloured *vanaspati* (7 red units)	Charcoal		Fuller's Earth	
	Furfurol test	Colour of *vanaspati*	Furfurol test	Colour of *vanaspati*
5 per cent	24·4	69·4	66·7	58·3
10 per cent	55·5	86·1	88·9	69·4

It will be seen that as far as the effect of adsorbents is concerned, both coloured *vanaspati* and *vanaspati* containing sesame oil, are almost equally affected. This point is very important but is overlooked by those who advocate the addition of sesame oil in preference to colouring *vanaspati* for detecting its presence in *ghee* as an adulterant.

Use of coloured vanaspati *to detect adulteration in* ghee.

(i) *Visual Detection.* The main object of colouring *vanaspati* is that if any of it is added to *ghee* its presence should be evident to the consumer. A colour intensity of seven red units is suggested as it is expected to provide a reasonably good safeguard without making *vanaspati* in any way unwelcome to the consumer. Adopting this colour standard, it has been possible to detect visually the

presence of even 5 per cent of coloured *vanaspati* in buffalo *ghee* and of 15 per cent in cow ghee. This is a great improvement over the existing routine chemical tests with which it is not possible to detect even up to 20—25 per cent of adulteration of *ghee*. This fact is well illustrated by the results given in Table V in which the data for the chemical analyses of adulterated *ghee* samples, using coloured *vanaspati*, are given along with the results of visual test.

TABLE V

Detection of adulteration of ghee with coloured vanaspati

Percentage of adulteration	Chemical analysis			Colour indicator	
	R.I. at 40°C.	Reichert value	Iodine value	Visual test	Lovibond Tintometer reading
I. *Buffalo ghee*—					
Control	41·8	36·6	27·7	—	R 0·1 Y 1·1
5 per cent	41·8	35·2	29·3	Suspicious	R 0·6 Y 1·1
10 ,,	42·0	33·5	31·3	Red (+)	R 0·9 Y 1·1
15 ,,	..	32·3	33·4	Red (++)	R 1·3 Y 1·1
25 ,,	43·8	29·3	37·9	Red (++++)	R 1·8 Y 1·2
II. *Cow ghee*—					
Control	43·3	25·1	36·7	..	R 1·3 Y 11·9
5 per cent	43·7	24·1	39·6	..	R 2·2 Y 11·9
10 ,,	44·4	23·0	41·7	Suspicious	R 2·4 Y 11·9
15 ,,	44·7	22·0	42·5	Red (+)	R 2·7 Y 11·9
25 ,,	45·7	20·3	48·2	Red (++)	R 3·3 Y 11·9

The above results show that the detection of adulteration of *ghee* with coloured *vanaspati* is much simplified. This is strikingly brought out in the case of buffalo *ghee* which has a high Reichert value. The results of visual test can be made quantitative by comparing the colour in Lovibond Tintometer.

(ii) *Detection by chemical methods.* The *ratanjot* dye gives a typical deep blue colour with alkalies. Depending on this reaction the following chemical tests have been evolved for detecting the presence of coloured *vanaspati* where visual detection may fail:

(*a*) When the adulteration of *ghee* is suspected to be 15 per cent and above, 10 gm. of the suspected sample is mixed with 25 ml. of alcohol and boiled. About 2 ml. of strong alkali is added to the mixture. In the presence of *ratanjot* the alcohol layer assumes a greenish blue colour.

(*b*) When *ghee* is made from butter coloured with annatto, the above test is not sensitive enough and it is necessary to first adsorb the colour on charcoal and then carry out the test. The method as finally adopted is as follows:

To 50 gm. of *ghee* about 2·5 gm. of adsorbent charcoal is added. The fat is heated repeatedly to 60 C., while being stirred. After about an hour the mixture is allowed to settle

when most of the charcoal goes to the bottom. The fat layer is then decanted off. The residual charcoal is warmed with 5 ml. of chloroform and filtered. The filtrate is collected in a white porcelain dish. The extraction is repeated two more time. The total filtrate is now freed from solvents on a water bath and a strong aqueous solution of alkali is added. The residue in the dish assumes a greenish blue tint in the presence of *ratanjot*. *Ghee* prepared from cow's milk or from artificially coloured butter does not show any change on the addition of alkali when subjected to similar treatment. By this test it is possible to detect the presence of even 5 per cent of added coloured *vanaspati*.

Toxicity of ratanjot gye. This has been systematically tested in experiments with rats and guinea pigs. It is found to be completely harmless. Even on heating to the frying temperature it does not acquire any toxic effects.

Summary

To safeguard trade in genuine ghee it is considered desirable that all *vanaspati* should be coloured. The colour should be attractive, very distinct from that of natural *ghee*, non-toxic, fairly stable to heat and light and should not impart any foreign taste and odour to *vanaspati*.

The use of *ratanjot* for this purpose is suggested. The intensity of colour recommended is seven red units.

As *vanaspati* is solely used as cooking medium its colouring is not likely to harm the legitimate interests of the *vanaspati* trade in any way.

Food cooked in *vanaspati* so coloured does not acquire any extraneous flavour, taste or colour.

Vanaspati coloured with *ratanjot* is found to preserve its colour at atmospheric temperature and in diffuse light such as are likely to be encountered under conditions during its storage and marketing.

Coloured *vanaspati* does not suffer in any way in its keeping quality.

While there are other tests such as the addition of sesame oil to *vanaspati* which can help to detect adulteration, colouring *vanaspati* provides the most direct and the simplest solution.

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COMPONENT OF FATTY ACIDS OF BUTTERFATS, HYDROGENATED GROUNDNUT OILS AND MUSTARD OIL

By V. R. Bhalerao, D. Venkatappiah, and C. P. Anantakrishnan, Imperial Dairy Research Institute, Bangalore

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NUMEROUS investigations have been reported on the detailed analysis of the cow butterfat, but not much information is available about the component acids of butterfats of Indian cow, buffalo, goat and sheep. Bhattacharya and Hilditch [1931] have studied the component acids of buffalo butterfat, but they have not taken into consideration the presence of lower unsaturated acids reported to be present in milk fats by Bowsworth and Brown [1933] and subsequently confirmed by Hilditch and Paul [1936], Longenecker [1937], Hilditch and Longenecker [1938] and others. Dhingra [1933] in his studies on the milk fats of Indian goat and sheep, has also omitted to estimate the lower unsaturated acids. Apart from these, no data has been published of the detailed composition of the component fatty acids of the butterfat. During some recent studies on the nutritive value of various fats carried out at this Institute a large number of fats were collected. This opportunity was taken to make a detailed study of the composition of buffalo, cow, goat and sheep *ghee*. Hydrogenated groundnut oils of m.p. 34·1°C., 37·0°C., and 39·0°C., and a sample of mustard oil were also available and they have been analysed in detail.

Experimental

Samples of genuine cow *ghee* from different breeds namely Kangayam, Sindhi, Ongole, Gir, Sahiwal, Hariana, Kangraj and Tharparkar were collected from different *ghee*-producing centres in India—Hosur, Madras, Charrodi, Dharwar, Karnal, Hissar, Montgomery, Delhi and Bangalore. Similarly six samples of buffalo *ghee* of Murrah breed from Hissar, Bangalore and Ambala, six samples of goat *ghee* from Hissar, Trichur, Agra, Anand and Chikkodi, six samples of sheep *ghee* from Montgomery, Anand, Sanand and Togh (Kohat) and six samples of mustard oil from Calcutta, Lahore and Cawnpore were collected. A composite sample prepared from each class of fat was used for the detailed analysis of the component fatty acids.

The groundnut *vanaspati* having m.p. 34·1°C., 37·0°C. and 39·0°C., were specially prepared and supplied for the study by the Hindustan Vanaspati Manufacturing Company, Bombay.

The method described by Smith and Dastur [1938] was adopted for the analysis of butterfats. About 289 gm. of butterfat was esterified by refluxing for 24 hours with methyl alcohol containing 5 per cent of sulphuric acid. The bulk of the unchanged methyl alcohol was removed by distillation and the mixed methyl esters in the residue was dissolved in ether and washed with water to remove mineral acid. The methyl esters were then distilled through an electrically heated and packed column similar to that described by Longenecker [1937] first under water pump and later at 2 mm. pressure until all the esters up to C_{14} or C_{16} had passed over into the distillate. Butyric acid present was estimated in (*a*) the methyl alcohol removed by distillation after the alcoholysis, (*b*) ether recovered from the esters and (*c*) the united aqueous portions by acidifying and steam distilling. The residual esters after preliminary fractional distillation were hydrolysed, submitted to lead salt separation and the two groups of higher acids (solid and liquid) re-esterified and further fractionally distilled.

The composition of the various fractions were calculated according to method discussed by Irving and Smith [1935] from the respective saponification and iodine values.

In the case of hydrogenated groundnut oil and mustard oil the initial esterification and the fractionation of the lower esters was omitted as they are known not to contain any lower component acids.

The general characteristics of the different fats examined are given in Table I. The percentage of different component acids as methyl esters in the four butterfats, three hydrogenated fats and mustard oil are shown in Tables II, III and IV respectively. These data have been summarized and expressed as percentages of fatty acids and molar percentages in Table V.

TABLE I

Analytical Constants of Fats used for detailed Analysis

Sample	Melting point	B. R. Reading at 40°C.	Sap. Value	Reichert Value	Polenske Value	Kirschner Value	Iodine Value
Cow butterfat	36·5	43·5	220·6	25·70	1·14	25·10	36·5
Buffalo butterfat	36·3	42·3	222·4	29·60	1·30	22·60	33·0
Goat butterfat	34·3	40·7	231·1	25·30	5·46	20·00	27·2
Sheep butterfat	35·4	41·9	232·7	27·30	3·90	20·30	29·7
Hydrogenated groundnut oil	34·1	54·6	199·0	0·00	0·00	..	78·7
Ditto	37·0	52·9	198·2	0·00	0·00	..	73·5
Ditto	39·0	51·7	197·4	0·00	0·00	..	69·8
Mustard oil	..	60·2	178·4	0·00	0·16	..	99·2

TABLE II

Component fatty acids of butterfats

Acids	Percentage as methyl esters							
	Lower	Solid	Liquid	Total	Lower	Solid	Liquid	Total
	(a) Cow Butterfat				*(b) Buffalo Butterfat*			
Saturated								
C_4	4·67	..	..	4·67	3·87	..	..	3·87
C_6	0·46	..	..	0·46	1·30	..	..	1·30
C_8	1·33	..	..	1·33	1·78	..	..	1·78
C_8	2·10	..	..	2·10	0·67	..	..	0·67
C_{10}	2·71	..	..	2·71	2·40	..	..	2·40
C_{12}	2·85	1·25	3·98	8·08	7·63	0·52	2·65	10·80
C_{14}	0·16	21·54	2·47	24·17	2·15	37·85	1·49	31·49
C_{16}	..	15·37	1·04	16·41	..	9·32	0·37	9·69
C_{18}	..	1·04	..	1·04	..	0·97	..	0·97
C_{20}								
Total	14·30	39·20	7·49	60·99	19·80	38·66	4·51	62·97
	(a) Cow Butterfat				*(b) Buffalo Butterfat*			
Unsaturated								
C_{10}	0·29	..	..	0·29	0·22	..	..	0·22
C_{12}	0·27	..	..	0·27	0·19	..	..	0·19
C_{14}	0·33	..	0·59	0·92	0·59	..	..	0·59
C_{16}	..	..	2·58	2·58	0·31	..	2·99	3·30
Oleic	..	2·40	28·43	30·83	..	1·83	28·86	30·69
Linoleic	..	..	2·97	2·97	..	..	0·73	0·73
C_{20}-C_{22}	..	..	1·16	1·16	..	..	1·29	1·29
Total	0·89	2·40	35·73	39·02	1·31	1·83	33·87	37·01

TABLE II—*contd.*
Component fatty acids of butterfats

Acids	Percentage as methyl esters							
	Lower	Solid	Liquid	Total	Lower	Solid	Liquid	Total
		(*c*) *Goat Butterfat*			(*d*) *Sheep Butterfat*			
Saturated								
C_4	4·13	..	..	4·13	4·21	..	..	4·21
C_6	0·27	..	..	0·27	0·39	..	..	0·39
C_8	3·42	..	..	3·42	3·72	..	..	3·72
C_{10}	8·72	..	..	8·72	7·61	..	..	7·61
C_{12}	4·40	..	0·14	4·54	3·95	..	..	3·95
C_{14}	3·43	2·69	3·66	9·78	4·43	4·20	4·0	12·63
C_{16}	0·96	25·92	1·56	28·44	0·66	25·86	1·71	28·23
C_{18}	..	10·42	0·86	11·28	..	7·38	0·81	8·19
C_{20}	..	1·28	..	1·28	..	0·52	..	0·52
Total	25·33	40·31	6·22	71·86	24·97	37·96	6·52	69·45
Unsaturated								
C_{10}	0·13	..	..	0·13	0·31	..	..	0·31
C_{12}	0·28	..	..	0·28	0·20	..	..	0·20
C_{14}	0·16	..	0·67	0·83	0·20	..	0·39	0·59
C_{16}	0·05	..	1·88	1·93	0·10	..	2·95	3·05
Oleic	..	2·05	20·71	22·76	..	2·15	21·67	23·82
Linoleic	..	..	0·91	0·91	..	..	1·74	1·74
C_{20}-C_{22}	..	..	1·14	1·14	..	..	0·84	0·84
N. S.	..	..	0·16	0·16	..	..	..	..
Total	0·62	2·05	25·47	28·14	0·81	2·15	27·59	30·55

TABLE III
Component of fatty acids of Hydrogenated Groundnut Oils

Acids	Solid	Percentage as methyl esters	
		Liquid	Total
		Melting point—34·1°C.	
Saturated			
C_{16}	7·87	1·18	9·05
C_{18}	6·11	..	6·11
C_{20}	2·30	..	2·30
C_{22}	2·88	..	2·88
C_{24}	1·28	..	1·28
Total	20·44	1·18	21·62
Unsaturated			
Oleic	6·25	58·04	64·29
Linoleic	..	9·97	9·97
C_{20}-C_{22}	..	3·36	3·36
N. S.	..	0·76	0·76
Total	6·25	72·13	78·38
		Melting point—37·0°C.	
Saturated			
C_{16}	7·68	1·18	8·86
C_{18}	9·81	..	9·81
C_{20}	2·43	..	2·43
C_{22}	3·11	..	3·11
C_{24}	1·30	..	1·30
Total	24·33	1·18	25·51

TABLE III—*contd.*

Component of fatty acids of Hydrogenated Groundnut Oils

Acids	Solid	Percentage as methyl esters	
		Liquid melting point 34·1°C.	Total
		Melting point—34·1°C.	
Unsaturated			
Oleic	8·50	52·71	61·21
Linoleic	..	8·57	8·57
C_{20}-C_{22}	..	3·97	3·97
N. S.	..	0·74	0·74
Total	8·50	65·99	74·49
		Melting point—39·0°C.	
Saturated			
C_{16}	5·01	4·04	9·05
C_{18}	12·52	..	12·52
C_{20}	2·54	..	2·54
C_{22}	3·46	..	3·46
C_{24}	1·30	..	1·30
Total	24·83	4·04	28·87
Unsaturated			
Oleic	14·79	45·02	59·81
Linoleic	..	6·40	6·40
C_{20}-C_{2}	..	4·02	4·02
N. S.	..	0·90	0·90
Total	14·79	56·38	71·13

TABLE IV

Component of fatty acids of mustard oil

Acids	Solid	Percentage as methyl esters	
		Liquid	Tota
Saturated			
C_{14}	0·63	0·65	1·28
C_{16}	..	..	..
C_{18}	..	..	..
C_{20}	..	..	..
C_{22}	1·03	0·02	1·05
C_{24}	..	0·96	0·96
Total	1·66	1·63	3·29
Unsaturated			
Oleic	5·39	21·51	26·90
Linoleic	..	16·42	16·42
Linolenic	..	1·88	1·88
Eureic	5·11	46·40	51·51
Total	10·50	86·21	96·71

TABLE V

Percentage by weight and molar percentage of the component acids of milk fats, hydrogenated groundnut oil, and mustard oil

Acid	Butterfats								Hydrogenated g.n. oil with m.p.							
	Cow		Buffalo		Goat		Sheep		34·1°C.		37·0°C.		39·0°C.			
	Weight	Molar	Weight	Molar	Weight	Molar	Weight	Molar	Weight	Molar	Weight	Molar	Weight	Molar	Weight	Molar
(*Saturated*)																
Butyric	4·2	11·3	3·5	9·5	3·8	9·7	3·9	9·3	..	..	..	..	..	..	..	..
Caproic	0·4	0·3	1·2	2·5	0·3	0·6	0·4	0·7	..	..	..	..	..	..	..	..
Caprylic	1·3	2·1	1·7	2·8	3·4	5·3	3·6	5·7	..	..	..	..	..	..	..	..
Capric	2·1	2·9	0·7	1·0	8·5	11·1	7·5	9·8	..	..	..	..	..	..	..	..
Lauric	2·7	3·3	2·4	2·9	4·5	5·5	3·9	4·4	..	..	..	..	..	..	..	..
Myristic	8·1	8·5	10·7	11·1	9·8	9·7	12·6	12·6	..	..	..	..	..	..	1·3	1·8
Palmitic	24·2	22·8	31·5	29·2	28·5	25·2	28·3	25·1	9·1	10·00	8·9	9·8	9·1	10·0	..	..
Stearic	16·6	13·9	9·8	8·2	11·4	9·1	8·3	6·6	6·2	6·2	9·9	9·8	12·6	12·6	..	..
Arachidic	1·0	1·8	1·0	0·7	1·3	1·0	0·5	0·4	2·3	2·2	2·4	2·3	2·6	2·4	..	..
Behenic	..	..	..	..	..	..	..	..	2·9	2·5	3·2	2·7	3·5	3·0	1·1	1·0
Lignoceric	..	..	..	..	..	..	..	..	1·3	1·0	1·3	1·0	1·3	1·0	1·0	0·8
Total	60·6	66·4	62·5	67·9	71·5	76·8	69·0	74·6	21·8	21·9	25·7	25·6	29·1	29·0	3·4	3·6
(*Unsaturated*)																
Decenoic	0·3	0·4	0·2	0·3	0·1	0·1	0·3	0·4	..	..	..	..	..	..	..	..
Do-decenoic	0·3	0·3	0·2	0·2	0·3	0·3	0·2	0·2	..	..	..	..	..	..	..	..
Tetra-decenoic	0·9	0·9	0·6	0·6	0·8	0·8	0·6	0·6	..	..	..	..	..	..	..	..
Hexa-decenoic	2·6	2·4	3·3	3·1	1·9	1·7	3·1	2·7	..	..	..	..	..	..	..	..
Oleic	31·1	25·2	31·2	26·3	23·3	18·8	24·2	19·5	64·8	64·8	61·7	61·9	60·5	60·5	27·2	29·8
Linoleic	3·0	2·5	0·7	0·6	0·9	0·7	1·8	1·4	10·0	10·1	8·6	8·7	6·5	6·6	16·6	18·2
Linolenic	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1·8	2·0
C_{20}-C_{22} unsaturated	1·2	0·9	1·3	1·0	1·1	0·8	0·8	0·6	3·4	3·2	4·0	3·8	4·1	3·9	..	..
Eureic	..	..	..	..	..	..	..	..	..	..	..	..	..	..		46·4
Total	39·4	33·6	37·5	32·1	28·4	23·2	31·0	25·4	78·2	78·1	74·3	74·4	70·9	70·0	96·6	96·4

Discussion

From the analytical data in Table I it will be seen that goat and sheep butterfats have almost similar characteristics to those of cow and buffalo butterfats. Goat and sheep butterfats have a high Polenske value and low iodine value. In the case of groundnut *vanaspatis* with a higher degree of hydrogenation as indicated by the melting point, there is a gradual decrease in the B. R. index and iodine value. The mustard oil is characterised from the other fats by a very high iodine value, and low saponification value, indicating the presence of glycerides of high molecular weight fatty acids.

The amount of butyric acid in cow butterfat as seen in Tables II and V is higher than in other butterfats in which the molar percentage of this acid is almost equal. The values for C_6-C_{10} acids in goat and sheep *ghee* confirm the high Polenske value already described in Table I. The data given by Dhingra [1937] and Hilditch and Jasperson [1944] are in agreement with present results. The percentage of lauric acid is also higher in the butterfats of goat and sheep. With regard to percentage of stearic acid, it is seen that the proportions in buffalo, goat and sheep butterfats are almost similar, while cow butterfat is richer by about 6·0 per cent in stearic acid content. The sum of myristo-palmi-to-stearic group acids is, however, constant in all the butterfats. The total of all the acids up to C_{14} is higher in case of goat (31·5 per cent, and sheep 32·9 per cent) milkfats as compared to cow (20·3 per cent) and buffalo (21·2 per cent) milkfats. This is mainly caused by the high amount of capric and lauric acids in goat and sheep butterfats. Arachidic acid is almost similar in all the fats.

The percentage of the lower unsaturated acids C_{10}-C_{16} in the butterfats is confirmed in the present study [*cf.* also Acharya and Banerjee, 1946]. It is found that they are distributed in almost the same proportion in all the butterfats. The percentage of oleic acid is similar in cow and buffalo butterfats, while in goat and sheep butterfats, the proportion is found to be much less as will be seen from Table V. This is in close agreement with the iodine values of the samples. There are marked variations in linoleic acid content of different butterfats. However, Hilditch has shown that the amount of this acid in butterfat varies with the diet of the animals as it increases if the animals are given plenty of green grass. It is found from the data that unsaturated acids higher than C_{18} are also present. No attempt was made to examine this fraction in detail and they have been grouped as C_{20}-C_{22} unsaturated acids. The proportions of this fraction in different butterfats were found to be almost similar.

Our results for palmitic and stearic acids in buffalo *ghee* agree closely with those of Acharya and Banerjee [1946]. The amount of oleic acid in the sample analysed here is much higher (26·3 per cent molar) than the value (17 per cent molar) shown by the above workers. These workers, however, have not given detailed results and as such no strict comparison can be made.

Our results for the major component acids of goat and sheep butterfats agree with those of Dhingra [1933]. It is also found (Table V) that the proportions of different acids in sheep butterfats are almost similar to those of goat butterfat.

The results of the analysis of hydrogenated groundnut oils of m.p. 34·1°C., 37·0°C. and 39·0°C. are represented in Table III. It is seen from those results that as the melting point increases from 34·1°C. to 39·0°C., there is a gradual increase in the content of stearic acid from 6·2 to 12·6 per cent. Hydrogenation, however, does not seem to affect palmitic acid, arachidic, behenic, and lignoceric acid contents. Obviously the total amount of saturated acids progressively increases with the rise in melting point. The percentages of oleic and linoleic acids on the other hand, gradually decrease. The net result of hydrogenation is the conversion of C_{18} unsaturated acids to C_{18} saturated acids.

The results of the composition of Indian mustard oil are presented in Table IV. The results closely agree with those reported by Sudborough *et al* [1926]. Mustard oil differs from common edible oils in that it contains a very high proportion of eureic acid (51·0 per cent). The oleic and linoleic acids are present to the extent of 29·8 per cent and 18·2 per cent respectively. Thus the total unsaturated acids in the mustard oil are 96·4 per cent, the remaining being made up of small quantities of myristic, behenic and lignoceric acids.

Summary

(1) Detailed analysis of fatty acids of cow, buffalo, goat and sheep butterfats, hydrogenated groundnut oils of melting point 34·1°C., 37·0°C., and 39·0°C. and mustard oil have been carried out.

(2) The cow and buffalo butterfats are found to be similar in composition and differ from goat and sheep butterfats, in that the latter contain more of caprylic, capric, and lauric acids and less of oelic acid. The presence of unsaturated acids lower than C_{18} in the butterfats of buffalo, goat and sheep have also been confirmed.

(3) In case of hydrogenated samples of groundnut oil, with the increase in the melting point there was a gradual lowering in the content of oleic and linoleic acids and a corresponding increase in stearic acid.

(4) The chief component fatty acids of mustard oil are found to be eureic acid (51·0 per cent), oleic acid (29·8 per cent) and linoleic acid (18·2 per cent).

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VITAMIN C CONTENT OF THE MILK OF SOME IMPORTANT BREEDS OF INDIAN CATTLE

By KERALA VARMA AND T. M. PAUL, Indian Dairy Research Institute, Bangalore

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A CONSIDERABLE amount of work has been done abroad to study the vitamin C content of milk and the influence of various factors like feed, season of the year, etc., on it. Riddel *et al* [1935] observed that the vitamin C content of milk was not subject to changes in the diet of the cow. Whitnah and Riddel [1937] studied the various factors affecting the vitamin C content of milk. They noticed that it was low in the early stages of the lactation; also that there was a rise from October to December, a fall from December to February and again a rise in February to March, but they could not correlate this fluctuation with any change in feed or any other factor and hence they thought it merely accidental. Holmes *et al* [1940] observed an increase in the first month, although the increase was not of much practical significance. Bartlet *et al* [1938] however found that the vitamin C level was very variable but was highest in the milk of cows fed on sprouted maize. Holmes *et al* [1941] have also recorded certain fluctuations which they correlated with advance in pregnancy. Here again, they found some difference from breed to breed, and there was a gradual fall in the vitamin content as pregnancy and lactation advanced. Karl Guggenheim [1940] observed a peak in the first half of the year, reaching the maximum in June-July which had no relation with feed. Thus the general conclusion is that feed has little effect on the vitamin C content, while breed, stage of lactation, etc., have some effect.

In India comparatively little work has been done on the vitamin C content of milk. The few investigations that have been made give the impression that the milk of Indian cows and buffaloes is rather poor in vitamin C content as compared to the milk of the European breeds. Ghosh and Guha [1935] have reported the vitamin C content of cows milk as 0·007 mg/ml. Chakravarthy [1935] has also recorded the same figures while Ranganathan [1935] got a still lower value of 0·004 mg/ml. Ray *et al* [1941] found the average vitamin C content of milk to be 0·0194 mg. per ml. with a maximum of 0·0223 and minimum of 0·0133 mg. per ml. Even this is lower than the figures reported for the milk of European breeds. Kothavala and Gill [1943] alone report the vitamin C content of the milk of Indian cattle to be comparable to those recorded by western workers. Such great difference in the values for vitamin C content of milk might possibly be due to the difference in the method of collection of the samples, part of the vitamin C being destroyed in cases where the milk has been exposed to light or metal surfaces. However, no conclusive data are available regarding the vitamin C content of the milk of the different breeds of Indian Cattle, or the extent of variations in the vitamin C content of their milk due to breed, season of the year and stage of lactation and hence the present work was undertaken.

EXPERIMENTAL

Five animals were selected from each of the following breeds maintained at this Institute farm: Sindhi, Gir, Tharparkar, cross-bred cows and Murrah buffaloes. The animals were of almost the same age and were in the first or second week of the particular lactation. The usual concentrate ration of the institute herd consisting of a mixture of wheat bran, gram, gram husk and groundnut cake, mixed in the proportion of 4·0 : 1·5 : 2·0 : 2·5, was fed to the animals at the rate of 1 lb. for every two pounds of milk yielded by the animal with a minimum of 3 lb. The roughage fed consisted of 55 lb. green grass (mixture of Guinea and Napier grasses) and 3 lb. of ragi straw for cows and 70 lb. of the same mixture of grasses and 3 lb. of ragi straw for buffaloes. Individual milk samples were collected in the morning in glass bottle coated outside with a thick layer of Japan black so that there was no chance of any action of light on the vitamin C content of the samples. Immediately the samples were analysed for their vitamin C content by the method of Bessey and King [1933]. To 20 ml. of milk, 5 ml. of 20 per cent trichloracetic acid were added; the mixture was then shaken well and filtered. Five millilitres of the clear filtrate was titrated against a standard solution of 2 ; 6

dichlorophenol indophenol and vitamin C expressed as mg. of ascorbic acid per litre of milk. From a few preliminary trials it was found that fresh milk collected in dark bottles contained only a very negligible amount of reversibly oxidised ascorbic acid. The same observation was made by Ray *et al* [1941]. Hence only the reduced ascorbic acid was estimated in the experimental samples. The estimation of the vitamin was carried out every week for about 12 months when most of the animals went dry. The average vitamin C content of the milk of each breed for each month is calculated and presented in Table I.

TABLE I

Average vitamin C content in mg. per litre of the milk of different breeds

Month	Sindhi	Gir	Tharparkar	Cross-bred	Murrah	Herd average for the month (Cow and buffalo)
November	18·1	18·0	19·1	21·4	13·1	16·2
December	23·1	19·5	19·2	21·1	21·2	19·8
January	22·9	22·8	20·1	20·1	28·5	22·9
February	25·6	23·5	21·6	20·8	27·7	22·2
March	21·8	20·8	19·7	20·8	27·7	22·2
April	22·1	20·4	19·4	17·5	27·0	21·3
May	21·4	19·0	18·5	19·0	24·5	20·3
June	20·0	19·4	20·0	17·4	24·5	20·3
July	26·8	21·1	20·0	20·7	26·3	23·6
August	28·4	24·6	19·6	20·2	25·2	23·2
September	30·0	25·6	..	22·9	..	26·2
October	..	26·4	..	23·2	..	24·8
Average for the lactation excluding the first month	24·2	22·1	19·8	20·4	25·6	22·6

DISCUSSION

It was noticed that in the case of all except cross-bred cows, the average vitamin C content of milk in the first month of lactation was slightly lower than the average value for each of the other months. These figures were also lower than the breed average or the herd average for the whole lactation. This is an agreement with the findings of Whitnah and Riddel [1937].

After the first month of the lactation, there was a gradual increase in the vitamin C content of the milk of all except cross-bred cows. This increase continued till the fourth month and thereafter the values began to decline till the end of the eighth month when an increase was observed which was kept up till the end of lactation. In the case of the cross-bred cows, from the very first month, the values for vitamin C declined gradually till the eighth month when an upward trend occurred and this was maintained till the end of the lactation.

The fluctuations observed in the vitamin C content of milk cannot be attributed to feed, since, the animals were receiving more-or-less the same feed throughout the experiment. Of the other factors that might influence the vitamin C content one is the season. In the present investigation two peak levels were observed, one in February and a still greater one in September, but in view of the more or less uniform climate of Bangalore, it may be reasonable to attribute these changes in vitamin C content of milk to lactational changes only.

It will be observed that breed variations in the vitamin C content of milk are quite marked. The average vitamin C content of the milk of the different breeds of Indian cattle as observed in the present investigation compares very favourably with the figures reported by foreign workers for the milk of European breeds.

Individual variations are observed in the vitamin C content of milk. In the case of the milk of Sindhi cows figures ranging from 21·9 mg. to 48·8 mg. of ascorbic acid per litre have been observed, but generally the range is much smaller. In the case of the other breeds also more or less the same

degree of variation was observed in the vitamin C content of milk, but in the case of cross-bred and Tharparkar cows the values for vitamin C fluctuated within narrow limits.

SUMMARY

(1) The vitamin C content of the milk from Sindhi, Gir, Tharparkar and cross-bred cows and Murrah buffaloes have been studied for one complete lactation.

(2) The average values obtained for the milk of the Indian cattle compare very favourably with the figures reported for European breeds.

(3) The vitamin C content of the milk was low in all the breeds in the first month of lactation; peaks were observed the fourth month and towards the end of lactation.

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INVESTIGATIONS ON FAMINE RATIONS—ENTRAILS AS A PROTEIN SUBSTITUTE FOR CATTLE

By N. D. KEHAR AND R. CHANDA, Animal Nutrition Section, Imperial Veterinary Research Institute, Izatnagar

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EVERY attempt at increasing the productive capacity of livestock in India is stifled by the quantitative shortage and qualitative deficiency of animal feed. According to a recent estimate the available concentrates are sufficient only for 29·1 per cent. of the adult cattle population. This does not take into account the requirement of goats, sheep, equines and the poultry, nor even the growth and productive requirements of bovines. Efforts have been made to meet this quantitative shortage by exploiting hitherto unutilized sources of food. The observations reported in this article relate to the use of entrails as a source of protein for cattle.

Although by-products of slaughter houses, like tankage, blood meal and fish meal, have been used for feeding swine, poultry and cattle, we have not come across any reference from the available literature where entrails have been used as a part or total substitute of protein in the ration of cattle. As observed by us in most of the small and average sized slaughter houses in this country entrails are regarded as waste material.

EXPERIMENTAL

The entrails of healthy animals consisting of the entire gastro-intestinal tracts of buffaloes, cows and bullocks were collected, washed of their contents and salted. The material was then heated in an autoclave for six hours between 115-120°C. at 15 lb. pressure. It was minced and dried for 24 hours in an electric oven at 80°C., cooled and then crushed to the size of a pulse grain and stored.

The chemical composition (on dry matter basis) of the entrails along with that of other slaughter house by-products is given in Table I for comparison.

TABLE I

Chemical composition of entrails and other slaughter house by-products

Material	Crude protien	Ether extract	Fibre	Nitrogen-free extract	Total ash	Ca	P
Entrails	76·13	13·78	1·03	1·49	7·57	0·162	0·396
Tankage or meat meal (best grade)	66·50	9·55	1·50	1·63	20·82	6·74	3·71
Blood meal	90·12	1·32	1·43	2·96	4·17	0·362	0·285
Fish meal (best grade)	72·10	7·35	0·76	3·90	15·89	4·58	3·31

In feeding this material to cattle it was found that unless the animals were very hungry they were not attracted by it. Entrails were then mixed with the oil cake and grain mixture in the ratio of 1 to 5 and gradually the quantity of entrails was increased. In this way the entire concentrates were replaced by entrails in about a month's time. The material was then fed to the experimental animals as a sole source of digestible protein for 10 weeks. The experimental ration consisted of 40 gm. of dry entrails and 2 lb. of wheat straw per 100 lb. body weight. The effect of feeding entrails on live-weight is given in Table II. It will be observed that the adult animals maintained their weight as if they were on the Institute scheduled ration.

TABLE II

The effect of feeding entrails on live-weight

Date	Animal No. 61	Animal No. 193	Animal No. 334	Remarks
8th September 1944	245	318	224	30 gm. of entrails and 150 gm. of groundnut cake
15th September 1944	240	312	220	50 gm. of entrails and 100 gm. of groundnut cake
22nd September 1944	236	290	218	75 gm. of entrails and 50 gm. of groundnut cake
29th September 1944	236	294	220	Entrails only
6th October 1944	238	290	216	"
13th October 1944	244	290	224	"
20th October 1944	240	308	228	"
27th October 1944	250	308	224	"
3rd November 1944	248	304	220	"
10th November 1944	244	318	224	"
17th November 1944	240	318	224	"
19th November 1944	244	312	224*	"
29th November 1944	248	316	222*	"
6th December 1944	248	316	226	"

* Metabolic experimental period.

A metabolism experiment was conducted during the latter part of the period of observations on three Kumaoni bullocks by the procedure detailed in an earlier publication [Kehar, 1944]. The digestibility coefficients of the whole ration and of entrails only are given in Tables III and IV respectively. In Table V is set out Nitrogen, Calcium and Phosphorus balances.

TABLE III

Digestibility coefficients of whole ration (entrails and wheat straw)

Animal No.	Dry matter	Organic matter	Crude protein	Ether extract	Fibre	Nitrogen-free extract	Total carbo-hydrates
61	51·7	54·7	47·2	57·3	61·3	48·9	55·2
193	51·8	54·8	45·8	56·2	61·2	49·3	55·4
334	59·1	61·0	48·5	58·9	68·0	55·8	62·0
Mean	54·2	56·8	47·2	57·2	63·5	51·3	57·3

TABLE IV

Digestibility coefficients of entrails

Animal No.	Crude protein	Ether extract
61	79·4	95·6
193	78·5	94·8
334	79·9	97·8
Mean	79·3	96·1

TABLE V

Nitrogen, calcium and phosphorus balances

Animal No.	Intake from entrails (gm.)	Intake from wheat straw (gm.)	Total intake (gm.)	Faecal excretion (gm.)	Urinary excretion (gm.)	Total excretion (gm.)	Balance (gm.)
			Nitrogen				
61	11·94	8·15	20·09	10·60	3·58	14·18	+5·91
193	15·19	10·88	26·07	14·12	6·48	20·60	+5·47
334	11·94	7·74	19·68	10·15	4·76	14·91	+4·77
			Calcium				
61	0·16	5·04	5·20	4·82	1·01	5·83	—0·63
193	0·20	6·73	6·93	6·08	1·34	7·42	—0·49
334	0·16	4·79	4·95	3·94	1·18	5·12	—0·17
			Phosphorus				
61	0·39	2·24	2·63	1·86	0·01	1·87	+0·76
193	0·49	2·99	3·48	2·02	0·02	2·04	+1·44
334	0·39	2·13	2·52	1·42	0·01	1·43	+1·19

It will be observed from Table V that nitrogen and phosphorus are in positive balance. The calcium balance is slightly on the negative side. If, however, we take into account the calcium intake of the drinking water (about 10 litres per day with a calcium content of ·05 gm. per litre), the small negative balance is approximately made up.

The biological value, total digestible nutrients and starch equivalent of entrails (per 100 lb. dry material) as compared to other concentrates are given in Table VI.

TABLE VI

The biological value, total digestible nutrients and starch equivalent of entrails as compared to other concentrates

Material	Digestible protein (lb.)	Starch equivalent (lb.)	Total digestible nutrients (lb.)	Biological value
Entrails	60·4	88·7	90·2	87·5
Tankage or meat meal (best grade)	61·2	83·2	84·6	..
Blood meal	77·5	81·8	83·2	79·5
Fish meal (best grade)	57·5	72·0	77·1	70·0

If, as given in Table VI, the digestible nutrients of entrails are compared with those of other products of animal origin, it will be observed that its digestible protein is superior to the best grade fish meal and equivalent to the best grade meat meal. In providing starch equivalent and total digestible nutrients, it is superior to meat meal, blood meal and fish meal of the best grades. Its biological value is superior to blood meal and fish meal of the best grade.

APPROXIMATE AMOUNT OF ENTRAILS AVAILABLE

It has been found that the dry material obtained from the entrails of an average sized cattle amounts to about 2 lb., and from that of a sheep or a goat about 0·4 lb. It has also been estimated

that from the slaughter houses in India 13·1 million pounds of the dry material can be obtained from cattle and 15·1 million pounds from sheep and goats annually. In addition 19·2 million fallen hides are obtained from dead animals per year. If we consider that 50 per cent. of these animals were disease free, we can get another 19·2 million pounds of the material from the dead animals. The total yearly production of 47·4 million pounds of the material can be fed to 142·2 million animals weighing 500 lb. for a day, as the total digestible protein supplement for maintenance. Expressed in other words, approximately four hundred thousand animals can be provided with a rich source of protein all the year round.

SUMMARY

Investigations have been carried out to find the possibility of feeding entrails as a total protein substitute for livestock.

Entrails which are a rich source of protein and fat have been fed to Kumaoni bullocks as a sole source of digestible protein for a period of 10 weeks. During the observation period the adult animals maintained satisfactory health and live weights.

The high digestibility coefficients of protein and ether extract (78·3 and 96·1, respectively) and the biological value, digestible protein and starch equivalent (87·5, 60·4 and 88·7, respectively) warrant its value as a high grade concentrate.

It has been calculated that if all the waste from the healthy dead animals is utilized, the digestible protein is sufficient to feed 142·2 million adult animals (500 lb.) for one day or approximately four hundred thousand adult cattle for the whole year.

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PROTECTIVE INOCULATION WITH DRY PIGEON-POX AND SHEEP AND GOAT-POX VACCINE

By D. A. MUNRO, Imperial Veterinary Research Institute, Izatnagar

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IN view of the well established efficacy of dry pigeon-pox vaccine for protecting fowls against fowl-pox, it was considered desirable to test the effectiveness of this method against similar infections of sheep and goats. The observations which follow throw light on the method of producing immunity with the use of a dry vaccine on both sheep and goats, as well as on fowls and pigeons. Amongst the most prominent methods of protective inoculation against variola infections, the one which has been found most suitable, and is now almost universally practised for the control of goat and sheep-pox, is the use of a sensitised vaccine as recommended by Bridré and Boquet [1913]. The conception of this method of prophylactic treatment owed its origin to Besredka's classical findings in the field of sensitised vaccines. These authors [1933] have since published statistical returns to show that nearly 25 million doses of the vaccine have been issued from the Pasteur Institute, Algeria, for use in various countries during the twenty years following its introduction and the results have been consistently satisfactory not only in so far as judged by the protection conferred on vaccinated sheep, but also by the degree of safety with which the latter could be allowed to come in contact with untreated animals.

In India, Viswanathan [1939] in Madras, reports his success in controlling an outbreak of sheep-pox by inoculating 2,388 sheep with the sensitised vaccine. The results obtained by him in goats, however, do not appear to have been satisfactory, for he writes: 'Out of 510 goats ovinated two died with symptoms of goat-pox, 20 developed severe generalized reaction and recovered and in the rest severe local reaction was present'.

Chadha [1939], in the North-West Frontier Province, tested the value of this method of vaccination on 77 sheep and found inflammatory reaction on the fifth day, with an elevation of body temperature 105°-107°F. In many cases the swellings reached the size of walnuts. The animals, however, eventually returned to normal health. While the introduction of the sensitised vaccine must be regarded as having registered a definite advance over the old method of 'ovination', a number of workers have referred to certain disadvantages of this method. Thus, Kolyali and Mavridis [1934] in Turkey, refer to the high cost of preparation of this vaccine and to the insufficiency of measures for its despatch, without loss of potency, to various parts of the country. Angeloff [1934] points out that the sensitised vaccine cannot be conserved and that, in spite of painstaking aseptic precautions in its preparation, there is a possibility of contaminating organisms developing during prolonged transit. Moreover it is not the case that animals treated by this method do not exhibit any symptoms. Thus, Suhaci [1939] states that, out of a total of 16,237 sheep vaccinated by the Bridré and Boquet method, nearly 73·75 per cent showed symptoms, though the latter were not generalized; 2·4-3·6 per cent of the animals showed nodules and many developed a transient lameness, while some showed depression and inappetence. Finally, as Bridré and Boquet [1933] themselves have pointed out, strains of sheep-pox virus vary in potency and this necessitates the use of varying quantities of serum for neutralizing a given quantity of virus. Kolyali and Mavridis [1934] later used sheep-pox virus treated with serum in the actual centre of the outbreak—a method which would appear to bear analogy with the serum-simultaneous method of inoculation against rinderpest. The virus was diluted in the proportion of 1 : 10 and four drops of the diluted virus were mixed with 5 c.c. of a strong anti-serum, these being left in contact for $1\frac{1}{2}$-$2\frac{1}{2}$ hours; each animal was inoculated intradermally with 0·25 c.c. of this mixture. Angeloff [1934], who has obtained excellent results with the simultaneous inoculation, mixed 0·1 gm. of fresh lymph with 10 c.c. of serum and injected 3 to 5 c.c. accordingly to the size of the sheep; the serum and lymph being mixed immediately before the injection. The drawback of this method, as Angeloff remarks, is that a large quantity of serum has to be arranged for.

The disadvantages, as indicated above, of using serumised vaccine led the writer to examine the desirability of reversion, in a suitably modified form, to the prophylactic treatment of sheep and goats by the use of vaccine alone. Ovination, which at one time was the exclusive method in most

parts of the world, has now almost been completely discarded and constitutes no more than an item of historical interest in literature on the subject, its serious drawback being, as is well known, that it sets up dangerous foci for spread of the disease owing to the severe reaction occurring in the inoculated animals. Similar remarks apply to the Borrel method [1930] which held the ground for many years before the introduction of sensitised vaccine, for, as remarked by Bridré and Boquet [1933], the virus employed in this method ' is not a vaccine ; its inoculation engenders sheep-pox transmissible by simple contact and the lesion, even if unique, is a source of active virus '. Blanc and Martin [1937] obtained inconstant results with the Borrel method in Morocean sheep when the latter were used as donors of the anti-serum required for use in this method. In India, Srikantiah [1936], states that he has employed the method successfully in 233 sheep. He, however, refers to the failure of its use by another worker in Madras during 1929-1930. Later, Srikantiah used what he designated the ' cutaneous method ' of preparing sheep-pox vaccine : the infective material was applied on the scarified portion of the skin of a healthy sheep and the epithelial layers of the resultant pustules were removed by scraping from the 13th to the 16th day. Two cubic centimetres of 1 : 3 glycerine solution was added to every gramme of the scrapings and the mixture preserved in cold storage, one part of the vaccine being diluted with two parts of saline at the time of despatch for field use. It would appear that two flocks out of three in which the vaccine was tested did not show any reaction and that eight of the sheep died ; this being ascribed to some unknown accident having occurred to the vaccine. It has, however, been observed by the present writer that pustules which are formed after four or five days contain the maximum amount of virus and that, as will be seen from what has to be stated later, vaccine in wet suspension, even when preserved in cold storage, quickly loses its potency. It is more than probable that these factors contributed wholly or partially to the failure to obtain successful results by the ' cautaneous method '.

The position in regard to protective inoculation against fowl-pox is more satisfactory, this being doubtless due to the widespread nature of the disease and the consequent availability of morbid material for experimentation, as also the obvious fact that birds are simpler and less expensive to experiment with. Of the more noticeable advances in this field, mention may be made of the introduction of pigeon-pox virus for use in fowls and of the so called ' stick method ' of vaccination. Reference may also be made to the findings made by Beller [1931] on the specific nature of fowl-pox lesions as distinguished from those due to deficiency of vitamin A. It is also noteworthy that, contrary to the generally accepted view, Kliger and Aschner [1931] have stated that fowl-pox and avian diptheria represent distinct disease entities, so that recovery from one does not confer protection against the other. If this statement is correct, then the percentage of success obtained with pigeon-pox virus against fowl-pox must have been substantially higher than what has been claimed for it in the past. It should, however, be mentioned that the relative value of fowl-pox and pigeon-pox virus still continues to be a subject of controversy. While, *a priori*, it would seem problematical whether a substantial degree of immunity is obtainable from the use of a heterologous vaccine, the actual experience of most workers points to a contrary conclusion. Thus James [1931], Glover [1931], Johnson [1932], Bierbaum [1935], Doyle [1935] and others refer to the highly satisfactory results obtained by them from its use, while, as will be seen from what has to be stated later, the present writer found it consistently effective in the experimental inoculation of fowls against pox. Michael [1932], Brunett [1933], and Lubbehusen and Ehlers [1934], record having found pigeon-pox virus to be of little value in protecting fowls, but as remarked by a number of later workers, their failures were in all probability due to faulty technique either in the preparation or in the application of the vaccine. It appears, however, generally agreed that the immunizing value of fowl-pox virus *per se* is superior to that of pigeon-pox virus, but that the use of the former is followed by a severe and sometimes fatal reaction, whilst egg production may be suspended altogether [James, loc. cit.]; Graham and Barger, 1936, Stafseth, [1931]. The danger of using fowl-pox virus is strikingly illustrated by an instance cited by Leyhausen [1933] in which considerable mortality occurred in a flock of chicks inoculated with a proprietary pigeon-pox vaccine containing traces of fowl-pox virus whilst no untoward symptoms occurred in birds which received pigeon-pox vaccine alone supplied at the same time by the same firm. The use of fowl-pox virus would thus appear to be fraught with real

danger and the writer therefore used only pigeon-pox virus in the experiments to be presently described.

According to Picard [1931], fowl-pox virus, by passaging through pigeons, may be attenuated and brought to a constant degree of virulence, so as to be suitable for inoculation with safety into fowls. Evidence produced more recently by Donatien and Lestoquard [1941], however, shows that the virus never reaches a fixed stage, but that it progressively loses its virulence, until the latter disappears altogether. An inquiry into the question of constancy of virulence of the virus was considered by the writer hardly profitable to undertake, for, apart from the results reported by the last two named workers, a biological standardization of the virus to ensure constancy did not, at least, in theory, seem possible. In fact, Goodpasture and Anderson [1940] have shown that immunity against fowl-pox is a function of the humeral antibodies and not, even partially of the epithelial cells, so that, it may be inferred that the virulence varies with the quantity of antibodies elaborated by individual birds. Moreover, it would seem open to question whether, in practice a state of constancy in virulence is really desireable if, as has been postulated by Basset [1935], the immunity produced is directly proportional to the pathogenicity of the vaccine, for this may imply that, for conferring a strong immunity, the titre of the virus has to be adjusted in relation to the general condition of the bird so that the reaction may not progress to a fatal one.

MATERIALS AND METHOD

The skin of the abdomen of a sheep was shaved and thoroughly cleansed with warm water and soap and dried. A one per cent suspension of sheep-pox virus in normal saline solution was now rubbed into the superficial epithelial layer of the skin which had been lightly scarified. After four or five days, material from the pustules was collected aseptically by scraping in a receptacle and was then desiccated *in vacuo* over phosphorus pentoxide or calcium chloride. The process of drying was carried out in the cold as high temperatures have a deleterious effect on the keeping quality of the vaccine. The material was now weighed and ground to a powder in a sterile mortar. It was then stored both in the form of wet suspension and in the form of dried powder at two temperatures, *viz.*, 42°F. and 98·6°F. The dry vaccine was prepared as follows before use. The material was rubbed to a smooth paste in a sterile mortar by the addition of a small quantity of 50 per cent glycerine saline. Sufficiently more of this diluent was now added to make the full quantity, which was then filtered through sterile muslin to remove extraneous matter and used the same day; 0·3 gm. of the dried vaccine suspended in the diluent was found sufficient for one hundred animals. A total of 110 animals were used in the present experiments and of these, 56 were sheep and 54 goats. The vaccinated animals were duly tested for immunity after an interval of 14 days. Controls were kept in each case.

In the case of pigeons, the feather follicles of both legs were plucked, and immediately thereafter a one per cent suspension of pigeon-pox virus in normal saline, was rubbed on to the denuded follicles. The crusts were collected from the 15th to the 21st day and the subsequent procedure was similar to that adopted in the preparation of sheep and goat vaccines as described above. The vaccinated birds were tested for immunity after 21 days by inoculating them with the specific virus. Controls were kept in each case.

EFFECT OF VACCINATION

Vaccination when carried out with a good sheep or goat vaccine usually resulted in a crop of vesicles at the site of inoculation resulting in a benign form of the disease. In a few sensitive patients the injection was followed by a period of dullness, loss of appetite, and fever, but this seldom lasted for more than a few days and the animal returned to normal after a short period of complete rest. The usual experience was, however, that no disturbance to health occurred in the animals that receive the protective inoculation. In some cases, when testing out brews of freshly prepared vaccine, the results were not as expected, the animal scarcely showing any reaction whatsoever. These animals when tested later with a potent virus failed to show any specific reaction, proving thereby that they were immune. The results are summarised in Table I.

TABLE I

Effect of vaccination in sheep and goats

Temperature	Duration of storage of vaccine	Vaccine used wet or dry	SHEEP							GOAT				
			Number of animals tested upon	Result of test in pustules	Number subjected to immunity test	Result of immunity test in pustules	Number of controls used	Reaction in controls	Number of animals tested upon	Result of test in pustules	Number subjected to immunity test	Result of immunity test in pustules	Number of controls used	Reaction in controls
	Immediate test within 96 hours	Wet	2	++	2	—	2	++	2	++	2	++	2	++
		Dry	2	— +++ +++	2	— —	2	— ++ +	2	++ +++ +++	2	+ —	2	— ++
	One month	Wet	2	— —	2	++ ++	2	+++ +++	2	— —	2	++ ++	2	++ ++
		Dry	2	+++ +++	2	—	2	++	2	+++ +++	2	++	2	++
	Two months	Wet	2	— —	2	++ +	2	+++ +++	2	— —	2	++ +	2	++ —
		Dry	2	+++ +++	2	+	2	+++	2	— —	(c)		2	—
42°F.	Three months	Dry	2	+++ ++	2	++ —	2	+++ +++ (a)	2	(b)	2	(b)	2	+ —(b)
	Four months	Dry	2	+++	2	—	2	+++	2	+++ +++	2	—	No control.	—
	Five months	Dry	2	+++ +++	2	—	2	++ ++	2	++ ++	2	—	2	++ +
	Six months	Dry	2	+++ +++	2	—	2	+++ +++	2	+++ +++	2	—	2	— —
98·6°F.	One month	Wet	2	— —	2	++ ++	2	++ ++	2	— —	2 1	++ ++	2	++ ++
		Dry	2	—	1(c)		2	+++ ++	2	—	(c)	—	2	—
	Two months	Wet	2	— — —	2	— —	2	+ — —	2	— —	1 (a)	— —	2	+ + ++
		Dry	2	—	2	—	2	+++	2	—	2	++	2	—

+++ Denotes several pustules
++ Denotes few pustules
+ Denotes very few pustules
— Denotes no pustules
Thus the symbols — in a column denotes that one animal showed few pustules and the other no pustules
(a) and (b) mixed with contaminating organisms
(c) Died before test

In the case of pigeons and chickens a good local reaction occurs and this usually takes the form of an inflammation and swelling of the feather follicles. In adult fowls the reaction is less marked, and at times almost imperceptible. The results obtained with the dry pigeon-pox vaccine are summarised in Table II.

(*a*) *In sheep*

It will be seen from Table I that out of a total of 14 sheep inoculated with the 'dry' vaccine that had been preserved in cold storage for varying periods, 11 (78·5 per cent) did not react when tested later for immunity, though the controls in each case developed the usual pustules following the virus inoculation. A total of six sheep were inoculated with the wet suspension kept at 42°F. and only one of these reacted to the vaccine, showing the remarkable rapidity with which this form of vaccine loses its potency (Srikantiah, *ante*). The protective value of vaccine stored at 98·6°F. was tested on eight sheep and none reacted to the inoculation, irrespective of the fact whether the vaccine used was in wet suspension or in the dried form, though six of these were found susceptible when tested later for immunity. In spite of the small number of tests, these results were considered sufficiently representative to warrant the conclusion that this temperature was quite unsuitable for storage of the vaccine and further tests along this line were not therefore proceeded with.

(*b*) *In goats*

It will be seen that a total of 18 goats were subjected to immunity test after they had received the protective inoculation with vaccine preserved at the lower temperature and that only seven proved resistant to the test. Five of the corresponding controls, however, did not react to the virus inoculation, so that the results in these cases must be regarded as inconclusive. The results obtained with vaccine stored at 98·6°F. were in conformity with those obtained in sheep, for not one of the goats in this category reacted to the vaccine.

Kolayli, Mavridis and Ilhami [1933] have recommended the utilization of sheep scab in vaccination of goats and the use of variola virus of goats against pox of sheep, for in either case a benign form of the disease is produced by the vaccine and, furthermore, according to these authors, 'the virus of variola of goats is not transmitted by natural contagion from goat to sheep'. A series of experiments were carried out to test the value of this method of vaccination for both sheep and goats and the results are shown in Table III.

It will be seen from Table III that ten sheep were inoculated with the specific vaccine and eight (80 per cent) did not react when later tested for immunity. On the other hand 10 goats were inoculated with sheep-pox vaccine and only four (40 per cent) proved immune on test. The protective value of goat-pox vaccine was tested on ten goats and ten sheep and positive results were obtained in eight (80 per cent) and three (30 per cent) animals, respectively. These results justify the conclusion that though the two viruses are closely allied, neither of them can be used as a dependable agent for protecting the heterologous species.

(*c*) *In fowls*

It will be observed that the vaccine stored for the maximum period of six months at 42°F. remains fully potent for fowls and pigeons, whereas a marked deterioration takes place when the vaccine is kept at 98·6°F. Tests on the protective value of vaccine in the wet state were not carried out in this series of experiments for previous experience has shown that the wet vaccine issued to the field deteriorated rapidly. Besides as already mentioned, such vaccine had been found to be unsatisfactory in the case of sheep and goats. Brunett [1933] also refers to the gradual loss of virulence in pigeon-pox virus when the latter is suspended in glycerine saline and recommend its storage in the powdered form, being suspended in saline only when required for use.

INDICATIONS FOR THE USE OF THE VACCINES

Sheep and goat pox vaccines may be used in clean flocks when the disease is known to be in the immediate neighbourhood in order to limit the spread of infection. In districts where the disease

TABLE II

Effect of vaccination in fowls and pigeons

Temperature	Duration of storage of vaccine	Vaccine used	Fowls						Pigeons					
			Number of birds tested upon	Result of test in pustules	Number subjected to immunity test	Result of immunity test	Number of controls used	Reaction in controls	Number of birds tested upon	Result of test in pustules	Number subjected to immunity test	Result of immunity test	Number of controls used	Reaction in controls
	Immediate test within 96 hours	Dry	3	+++ +++ +++	3	— — —	2	+++ +++	3	+++ +++ +++	3	— — —	2	+++ +++
42°F.	One month	Dry	3	+++ +++ +++	3	— — —	2	+++ +++	3	+++ +++ +++	3	— — —	2	+++ +++
98·6°F.	One month	Dry	3	— — +++	3	— — —	2	+++ +++	3	— — —	3	+++ — ++	2	+++ +++
42° F	Two months	Dry	3	+++ +++ +++	3	— — —	2	+++ —	3	+++ +++ +++	3	— ++ —	2	+++ +++
98·6°F.			3	— — —	3	— — —	2	+++ —	3	— ++ ++	3	— — —	2	+++ +++
42°F.	Six months	Dry	3	— — —	3	— — —	2	+++ +++	3	+++ +++ +++	3	— — —	2 ..	+++ +++
98·6°F.			3	— — —	3	++ — +++	2	+++ +++	3	— — —	3	— +++ +++	2	+++ +++

++ Denotes inflammation and swelling of the feather follicles
+++ Denotes vesicles, pustules, and scab formation
— Denotes no reaction

TABLE III

Effect of dry vaccine on sheep and goats

Temperature	Duration of storage of vaccine	Vaccine used sheep or goat Dry	SHEEP						GOAT					
			Number of animals tested upon	Result of test in pustules	Number subjected to immunity test	Result of immunity test in pustules	Number of controls used	Reaction in controls	Number of animals tested upon	Result of test in pustules	Number subjected to immunity test	Result of immunity test in pustules	Number of controls used	Reaction in control
42°F.	One month	Sheep	2	+ +	2	— —	2	+ + + + + +	2	+ +	2	+ —	2	Not tested + + +
		Goat	2	+ + +	2	+ + + + +	2	+ + + + + +	2	+ + + + + +	2	(a) —	2	Not tested + + +
42°F.	Two months	Sheep	2	+ + + + + +	2	— —	2	+ + + + + +	2	+ + + + + +	2 2	— + +	2	+ + + + + +
		Goat	2	+ + + +	2	+ + + + +	2	+ + + + + +	2	+ + + + + +	2	— —	2	+ + + + + +
42°F.	Three months	Sheep	2	— +	2	— —	2	+ + + + + +	2	+ + + + +	2	+ + + + +	2	+ + + + + +
		Goat	2	+ + + + +	2	+ + + +	2	+ + + + + +	2	+ + + + + +	1	— (a)	2	+ + + + + +
42°F.	Four months	Sheep	2	— —	2	+ + + + +	2	+ + + + + +	2	— —	2	— —	2	+ + + +
		Goat	2	+ + + +	2	— —	2	+ + + + + +	2	+ + + + + +	2	— —	2	+ + + +
42°F.	Five months	Sheep	2	+ + + +	2	— —	2	Not tested + +	2	+ + + + + +	2	+ + + + +	2	+ + + + + +
		Goat	2	Died + +	1[a] (a)	—	2	Not tested + +	2	+ + + + + +	2	— —	2	+ + + + + +

Reading of notations same as Table I

(a) Died before test

has a seasonal incidence, inoculations may be carried out in good time beforehand, and not as an emergency prophylactic measure. It may also be used in clean flocks where the disease tends to linger on. On the other hand, inoculations are better not performed on healthy animals if there is no immediate danger, for, as already mentioned, a benign form of the disease is set up after vaccination, and a sick animal is likely to spread the disease to other animals which have not been inoculated. As for the fowl-pox vaccine, it should not be used in pigeons in a debilitated state, as the vaccine in such cases is capable of setting up the disease in a generalized form.

Summary

Sheep and goat-pox vaccines stored in the form of a dried powder remains potent for the purpose of protective inoculation for not less than six months, and similar results were obtained with a dried pigeon-pox vaccine. When stored in wet suspension, sheep and goat-pox vaccines lose their potency within a month. Sheep-pox vaccine has not proved to be a dependable agent for protecting goats, and goat-pox vaccine for protecting sheep.

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GEOGRAPHICAL AND SEASONAL INCIDENCE OF SURRA IN THE PUNJAB

By H. K. Lall, B.Sc., M.R.C.V.S., *Assistant Animal Husbandry Commissioner with the Government of India**

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(with five text figures)

Basu [1945] collected the data concerning surra in all species of animals under three headings, i.e., bovines, equines and others from the year 1942 to 1944 from various provinces and States, showing the intensity of infection in the various areas. From his figures the Punjab seems to be the worst affected area, followed by the United Provinces and Sind. In this note data for the last 14 years 1932-44) has been collected from the five divisions of the Punjab (Lahore, Multan, Rawalpindi, Ambala and Jullundur) and an effort has been made to work out correlation between rainfall (the averageof figures for last 14 years) and the incidence of surra, if any. Table I and Fig. 1* indicate that there is no direct relationship between rainfall and the number of surra cases. The number of surra cases is more or less constant. If anything there is a fall in the incidence** of the disease in heavy rainfall years. No doubt there is a seasonal occurrence of the disease, i.e., there is increase just after the rainy season, as can be seen from Fig. 2. The low incidence in heavy rainfall years may be due to the washing away of eggs and larvae of the insect vectors. The abnormal fall in the number of surra cases recorded in the years 1943 and 1944 is due to the fact that naganol was not available. This point is discussed in detail later.

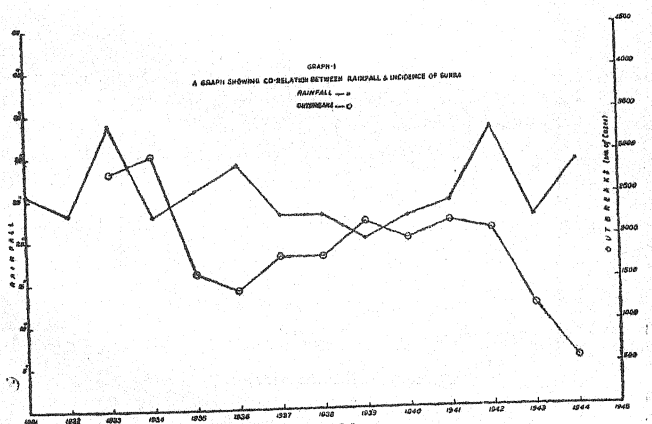

Fig. 1. Correlation between rainfall and incidence of surra.

Seasonal incidence. The figures for each month for the last 14 years have been computed and the average calculated. It will be seen from Table II and Fig. 2 that the number of surra cases start increasing in August and reach their zenith in October. The peak is reached in September in Ambala, Multan and Rawalpindi and in October in Lahore and Jullundur (see Fig. 3A and Table II). The figures available for Assam and the Central Provinces for the last two years also indicate a similar *seasonal curve.* On the other hand, in the seasonal curve drawn by Basu (ibid) on the basis of total figures, the peak is reached earlier in September then in our case, viz. in October. The separate figures for horses and camels were available for Jullundur division only in which case a separate figure has been drawn (Fig. 3B), which shows that the peak in the cases of the equines is reached in

October and in camels in September. These curves are similar to those for equines and others, obtained by Basu (ibid.) According to Basu the peak in bovines is reached in August, while from the data supplied by Zargar (personal communication) for the years 1940-1943, the peak in bovines in the Central Provinces is reached in September. This may be due to variable climatic factors such as earlier rainfall at one place as compared to the other.

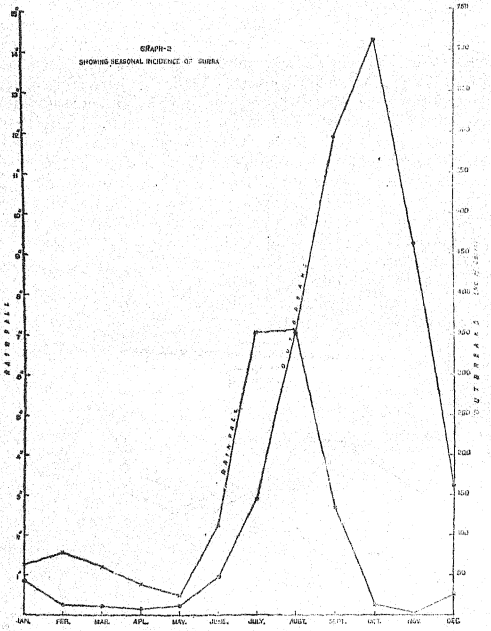

Fig. 2. Seasonal incidence of surra.

TABLE I

The number of surra cases recorded and the average yearly rainfall in the Punjab from 1932-1944

Years	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	1945
Number of recorded cases of surra in the Punjab.	..	2,824	3,051	1,638	1,419	1,933	1,891	2,276	2,056	2,208	2,186	1,260	583	..
Number of recorded cases of surra in Ambala Division.	..	..	..	..	..	977	702	773	1,191	593	1,362	435	258	327
Rainfall in Inches	23·21	33·70	22·87	25·80	28·79	22·93	22·93	20·06	22·64	24·31	33·10	22·57	28·86	..

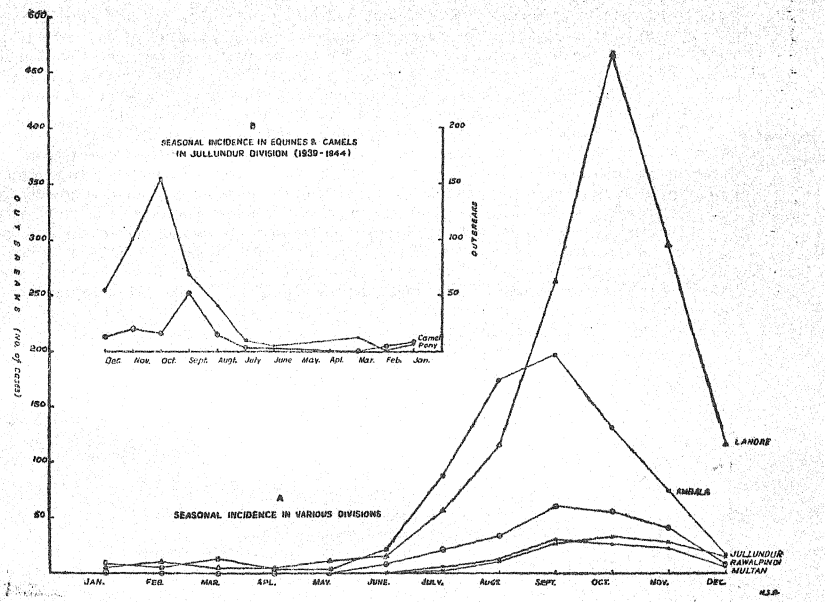

Fig. 3. Seasonal incidence of surra.

When the figures of incidence of surra are compared each year, it is seen that peak is reached in the same month each year, with occasional minor variations. The average rainfall has also been worked out monthwise on the basis of the rainfall figures of the last 14 years and plotted against the number of average outbreaks each month. These figures show that surra has no direct relation to rainfall as previously stated but its incidence is increased in the months usually after the summer monsoons in the Punjab.

It is known that trypanosomes feed by osmosis on the glucose of blood which is naturally mobilised from the store, i.e., the liver, and that there is hyperglycaemia in the early stages of trypanosome infection and hyperglycaemia in the latter stages when the trypanosomes usually disappear. Ray (personal communication) pointed out to the author that there is a seasonal variation in the blood sugar of equines. The seasonal incidence of the disease and seasonal variation in the quantity of blood sugar and the increase in the number of flies and other insects during the surra season throw some light on the epizootology of the disease. It seems from the histopathology of the disease studied on a few cases by Ray and Lall in 1944 (unpublished) that the lesions produced in chronic cases of surra are pathogonomics of sugar-glycogen metabolic disorders and its further study—specially of the endocrines—is likely to reveal some useful information.

Geographical distribution. A comparison has also been made on a geographical basis, i.e., the number of outbreaks per total equine and camel population have been examined in the various divisions of the Punjab, having different climatic conditions and rainfall. The number of surra centres, most of which are along the courses of the rivers, varies in each division (see Fig. 5). This might have been one of the reasons for variance in figures, but the analysis of outbreaks for each centre also shows the same result as obtained on the basis of average population. It will be seen from Table III that the

number of cases are fewer in the dry climate of Multan as compared to that of Ambala and Lahore where the climate is more humid and there are extensive low lying areas. The last factor seems to be the deciding factor, as far as surra is concerned. The total rainfall in Lahore and Rawalpindi and Ambala divisions is approximately the same, but there is a difference in the number of cases (on the average basis per 10,000 heads of equine and camel population) in each division. Jullundur, having a much heavier rainfall, has more or less the same incidence as Rawalpindi. The peak is reached in October in Lahore and Jullundur, in September in Ambala, Rawalpindi and Multan (see Fig. 3A); this is very probably owing to the larger camel population in these divisions, because in the case of camels peak is reached in September. In Ambala, which has proportionately a large camel population, it is noticed from the records that the majority of the surra infected animals brought to hospitals were camels. In the divisions where the camel population is considerable in proportion to equines, the peak is not well defined. It is seen from the monthly rainfall curves (the average of 1935—1943) of the various divisions (see Fig. 4) that the highest rainfall is in July in Ambala, Jullundur and Multan and in August in Lahore and Rawalpindi. There seems to be no obvious connection between the earlier period of rainfall and the peak period in this case. Winter rainfall due to winter monsoons is maximum in Rawalpindi. It would seem, however, the winter rainfall has no co-relation with the incidence of surra.

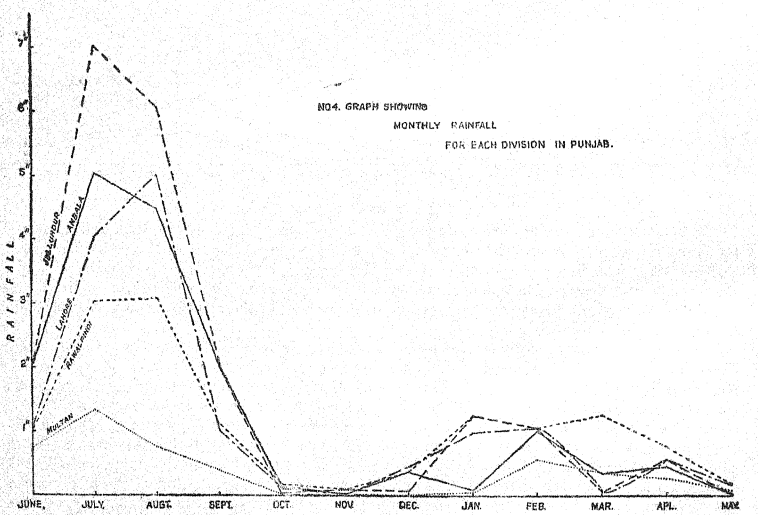

Fig. 4. Monthly rainfall for each division in the Punjab.

TABLE II

Monthwise incidence of surra of each division of the Punjab and average monthly rainfall

Months	January	February	March	April	May	June	July	August	September	October	November	December
Lahore (average number of cases).	27·5	11·5	7·1	7·1	12·1	18·6	67·1	115·5	27·2	475·2	298	115
Rawalpindi . . .	2·6	1·4	·36	·85	1·8	8·0	22·3	38·4	63·0	55·2	44·3	9·0
Multan	3·5	2·4	2·1	·57	·7	1·4	6·4	14·7	304	26·0	23·7	6·8
Jullundur . . .	1·6	2·2	·8	·0	·6	·4	3·0	11·4	27·0	36·6	28·6	15·0
Ambala	8·6	5·6	12·2	9·4	9·3	21·2	88·5	173	195	130	67	16·4
Total .	43·8	23·1	22·56	17·92	24·5	49·6	187·3	353·0	387·4	723·0	461·6	162·2
Rainfall . . .	1·27	1·57	1·21	0·79	0·50	2·25	7·07	7·15	2·70	0·28	0·07	0·52

Note discussed at the Seventh Meeting of the Animal Husbandry Wing held at Lucknow.
** The figures indicating surra incidence for Ambala division have not been included in this graph.
† Average is based on the figures of years 1933-44 in case of Lahore and Rawalpindi, 1937-45 in case of Ambala, 1937, in case of Multan and 1935-44 in case of Jullundur.

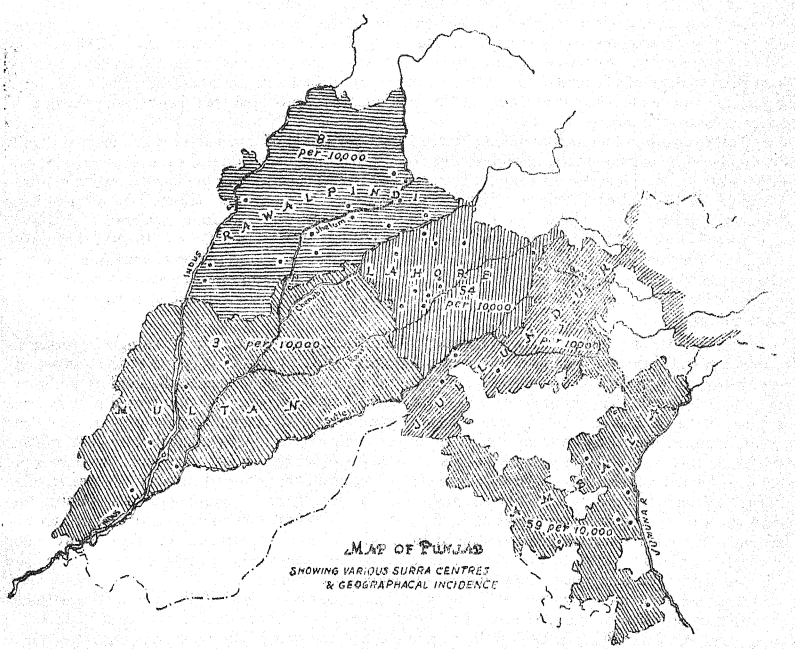

Fig. 5. Map of the Punjab showing various surra centres and geographical incidence.

TABLE III

Geographical incidence of surra in the Punjab

Divisions	No. of surra centres in each division	Total number of outbreaks	Average No. of outbreaks per year	No. of cases per centre	Population of equines (Horses, donkeys and camels)		Average No. of outbreaks per 10,000 animals	Average rainfall in inches (1931-44)
Lahore (1930-1944)	10	7,678	1,280	128	E C	221,732 13,417	54·4 (54)	24·47
Jullundur (1930-1944)	4	634	106	20·5	E C	91,349 54,051	7·2 (7)	44·21
Rawalpindi (1930-1944)	13	1,589	265	20·5	E C	278,941 54,317	7·9 (8)	23·20
Multan (1930-1944)	6	674	112	18·6	E C	302,604 113,415	2·7 (3)	8·47
Ambala (1930-1944)	8	4,354	726	90·7	E C	83,842 40,202	58·6 (59)	26·66

E= Equines. C = Camels.

† Punjab Census Report. (1940)
‡ Figures in brackets have been shown in the map.

The effect of chemotherapy on the incidence of surra. In the early part of disease investigation schemes extensive trials were made by the field staff of the provinces with tartar emetic, naganol alone and both combined in the treatment and prevention of surra. But, as late as 1936, Taylor Director of Veterinary Services, Punjab reported that the treatment of equine surra with naganol was not an unqualified success. In 1940 Walker reported that surra in horses could be cured both by naganol alone and the naganol cum tartar emetic method, provided treatment was applied in the early stage of the disease.

Tartar emetic, which was commonly in use for the treatment of camel and equine surra, gradually was replaced by naganol in the case of equines, but in the case of the camels its use remained circumscribed due to its high cost. In bovines, however, it has been successfully used as a curative and prophylactic in the United Provinces where it has been tried in the years 1939-1945 on a fairly large scale in a limited area, while successful curative effects are reported from Hyderabad (1944-45), Madras (1941-42, 1943, 1944) and Assam (1943-1944 and 1945). From Orissa (1940-41 and 1942), however, the results of treatment of bovine surra with tartar emetic were not so convincing; symptoms of shock were noticed in some of the treated cases, while some animals succumbed to the disease in spite of the treatment. In one case from Madras also ulticarial eruptions and symptoms of distress were noticed.

Naganol became very popular as a curative remedy in surra because of the single injection required; second injection being necessary in a few cases only. From the reports of the Disease Investigation Officers for 1939 to 1945, it seems that naganol has been successfully used as a curative in all parts of India. The prophylactic value attributed to it, however, can be accepted with reserve because of the lack of controls in most of the experiments undertaken. The supply of the drug became limited during the war and the stock in the country was nearly exhausted by 1942 when it was being sold at the exorbitant price of Rs. 50 a dose so that only a few race horse breeders could afford. Since 1942 there has been an enormous reduction in the recorded number of cases of surra in the various hospitals in the Punjab as can be seen in Fig. 2 and according to the information from the various veterinarians working in the hospitals. This reduction is a psychological phenomenon. The clients, having got accustomed to single injection treatment of naganol, were not inclined to revert back to tartar emetic which, in most of the cases, necessitated more than one injection and even then there were chances of relapse occurring.

Antrypol came into prominence during the War. It has been successfully used in the treatment as well as prevention of surra on a large number of ponies in Assam during the years 1942, 1943, 1944. In 1943-44 in Sind, in 1944-45 in Kashmir, good results were obtained by its use in camels in Sind the groups of camels injected with antrypol remained free from the disease. In the Central

Provinces, however, in 1945-46 extensive ulticarial eruptions lasting for 12 hours were reported as the after-result of antrypol treatment in the only case treated.

SUMMARY

The data of surra cases in the Punjab, for the year 1932-1944 has been analysed and its seasonal and geographical distribution discussed the study of the relevant records in the Punjab points to the facts that there is no quantitative relationship between the incidence of surra and the annual rainfall, and that the incidence of the disease always increases after rainfall, indicating the need of prophylactic treatment for one or two months following for summer monsoon.

There is a decline in the number of surra cases presented for treatment following the cessation of the use of naganol, it appears that owners will forego treatment rather than submit to one which causes some inconvenience; it, therefore, follows that simplicity of treatment should take precedence over the ultimate total effectiveness of the treatment or should at least be considered as equally important to it.

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A NEW METHOD FOR THE STUDY OF EXTENSION IN WOOL FIBRE

By P. N. Nanda, M.R.C.V.S., and Tarlok Singh, B. Sc. (Hons.), M. Sc., Government Livestock Farm, Hissar

(Received for publication on 6 May 1946)

(With plate III and one text figure)

STRENGTH and elasticity form two of the most important qualities of wool, as the strength and weaving properties of the fabric are dependent upon them. Whether a fabric is fine or coarse, it must handle elastic and lofty. The term elasticity, as applied to wool, denotes the power it possesses to assume its normal condition after being subjected to stress. Its exact determination may be made by the measure of the limit upto which the fibre extends when under stress.

For the measurement of strength in wool fibres, Oneill Hair Tester, originally devised by Charles Oneill [1863] is being used in this Farm Laboratory and it is described in detail in the *Textile Journal*. As regards extension, however, very little information is available. In the course of our experiments on the measurement of fibre strength, we were able to introduce a subsidiary contrivance within the apparatus without altering it in any way. With the help of this contrivance, it is possible to measure the maximum length to which a fibre or a portion of the fibre extends before it snaps.

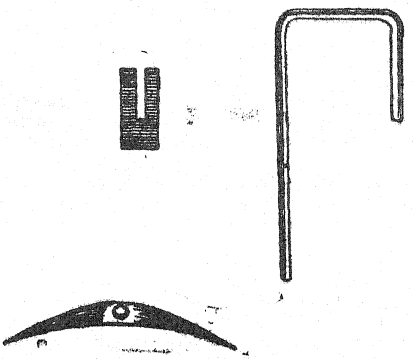
The contrivance, as shown in Figs. 1-3, consists of the following four parts :

(i) The support, a thin cylindrical piece of wood about half an in. long, having a longitudinal hole in the centre, which goes as deep as its middle (Fig. 1). The support is tied to the cork of the float of the tester with the help of a thread as shown in Plate III. The lower end of the support is made to remain above the level of the water when the float is freely and vertically suspended in it.

(ii) The rider consisting of a piece of fine metallic wire bent at right angles at both ends, one arm being longer than the other (Fig. 2). The shorter arm fits into the hole of the support while the longer arm carries the index at its end.

(iii) The index, a crescent shaped metallic piece, the inner curvature of which is the same as the curvature of the outer tube of the tester (Fig. 3). It has a hole in the middle in which the end of the longer arm of the rider is fixed. The index moves along the scale.

(iv) The scale marked in inches (1/10th) on an ordinary piece of white paper, which is rendered water-proof by giving it a dip in hot paraffin. The scale is pasted on the outer surface of the cylinder of the tester at its upper end.



Figs. 1-3. Parts Oneill Hair Tester 1—The support, 2—The rider, 3—The index.

Working

When the apparatus is in use, it operates as shown in Plate III. The fibre to be tested is mounted on paper mounts, the ends of the fibre being secured with sealing wax at a distance of one inch apart. This length is selected as a standard for comparative study. The paper is then fixed in the hooks provided in the apparatus. Along with the fibre, the rider is also put in the hole of the support. After fixing the upper fixed point of the apparatus, the initial reading at which the pointed end of the index stands is taken. Readings are taken upto two decimal places, the second place being estimated by judgment. Water is then allowed to flow out of the tester as a result of which pressure is exerted on the fibre and it extends. On account of the extension, the tube moves down, and with it the index also glides downwards along the scale. A careful watch is kept on the movement of the index. Just when the fibre breaks, reading at the scale is noted. The difference between the initial and the final readings on the scale gives the limit up to which the fibre extended per unit inch. The water taken out for breaking the fibre is measured in c.c. and the breaking load is calculated by the formula :

$$F = \frac{Vr^2}{2R - r^2}$$

where F is the breaking load,
V, the volume of water drawn,
r, the radius of the float,
R, the radius of the outer cylinder

As soon as the fibre breaks, the float suddenly falls down, with the result that the rider strikes against the edge of the outer cylinder and thus easily comes out of the hole in the support and remains suspended on the edge for use again.

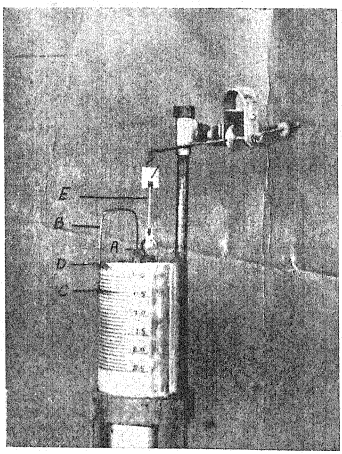
To ensure efficient working of this subsidiary device, a ring is fixed at the top edge of the outer tube just above the scale, through which the longer arm of the rider is made to pass, (Plate III). This ring serves the purpose of keeping the rider in one plane and does not allow it to go astray due to the free motion of the inner float, thus ultimately helping the index to glide smoothly along the scale. Care is necessary to see that the index does not touch the scale, otherwise the resulting friction will interfere with the movement of the index. Some distance should be left between the scale and the index. This can be done conveniently by adjusting the upper hook of the tester so as to suit the requirement before starting the experiment. Once the distance is adjusted, it will continue to work satisfactorily.

This device is claimed to be particularly suitable as it determines the breaking load and extension of the fibre at one and the same time. Gohachi Osumi and Etsuro Kato [1937] in developing their own tester, assert that a perfect tester intended to record the elongation of the fibre must be simple both in making and handling, and it must measure the elongation on a magnified scale. The method now described is not only simple, but also denotes the elongation on a magnified scale as the fibre length is taken as one inch standard and the scale being in one tenths of an inch, enables the worker to note down even a slight extension with the naked eye.

Preliminary trials

In order to test the accuracy and consistency of the results obtained by this method, preliminary trials were carried out only on true wool fibres (fine fibres) which are considered to be fairly uniform with regard to their structure and other characteristics such as length, circumference, strength, etc. in a particular wool sample. The trials were divided under two heads :

(*a*) *Trials by one observer.* Two samples from the shoulder region from ewe no. 477R (sample No. 1) and ewe no. 663 (sample No. 2) consisting of only wool fibres (other fibres having been removed from the samples by mechanical means and the classification confirmed by the Benzene test) were taken. The samples were freed from impurities by a bath in warm benzene. Sample No. 1 was then divided into several lots and from each lot a small sheaf of fibres was drawn and pooled together to form a composite sample. This was further treated in a similar way and the process repeated till a small representative sample was obtained. From this sample, six sets of ten fibres each were



Upper part of the Oneill Hair Tester showing the subsidiary contrivance.
A, Support; B, Rider; C, Index; D, Ring; E, Fibre

drawn at random, mounted on paper mounts, and tested for extension. Sample No. 2 was then handled in a similar way, making a total of 12 trials in both the samples. Results are given in Table I. Conclusions regarding the consistency of the data were drawn from these results by applying the analysis of variance according to the method suggested by Fisher [1941]. Assuming that every set of ten fibres represented the bulk population to which it belonged, and the fibres used in every set or trial were uniform as regards their characteristics, the results obtained can be considered as reliable.

TABLE I

Extension per unit inch

Sample No.	Fibre No.	1	2	3	4	5	6	7	8	9	10	Total
1	Trial No.											
	1	0·35	0·38	0·30	0·23	0·32	0·40	0·30	0·41	0·38	0·45	3·52
	2	0·20	0·40	0·29	0·29	0·40	0·31	0·40	0·41	0·31	0·36	3·37
	3	0·36	0·23	0·42	0·39	0·40	0·36	0·26	0·25	0·36	0·35	3·38
	4	0·40	0·42	0·38	0·41	0·45	0·48	0·43	0·35	0·37	0·34	4·03
	5	0·20	0·39	0·35	0·35	0·37	0·37	0·36	0·37	0·25	0·25	3·26
	6	0·33	0·29	0·41	0·30	0·42	0·35	0·43	0·35	0·40	0·39	3·67
	Total .	1·84	2·11	2·15	1·97	2·36	2·27	2·18	2·14	2·07	2·14	21·23
2	Trial No.											
	1	0·40	0·38	0·40	0·38	0·40	0·23	0·33	0·45	0·40	0·37	3·74
	2	0·45	0·43	0·35	0·40	0·42	0·46	0·50	0·44	0·39	0·40	4·24
	3	0·22	0·42	0·48	0·36	0·35	0·48	0·44	0·40	0·50	0·41	4·06
	4	0·39	0·42	0·34	0·40	0·45	0·40	0·40	0·37	0·34	0·37	3·88
	5	0·42	0·44	0·38	0·40	0·38	0·38	0·32	0·38	0·40	0·40	3·90
	6	0·40	0·45	0·44	0·40	0·40	0·44	0·40	0·47	0·38	0·42	4·20
	Total .	2·28	2·54	2·39	2·34	2·40	2·39	2·39	2·51	2·41	2·37	24·02

TABLE II

Analysis of variance—sample No. 1

Source	D.F.	S.S.	M.S.	Z
Between trials	5	0·0390	0·0078	..
Within trials	54	0·2008	0·0037	0·373
Total .	59	0·2398	0·0040	..

The value of Z being non-significant, shows that there is homogenity in the data of Sample No. I with a total variance of 0·0040.

TABLE III

Analysis of variance—sample No. 2

Source	D.F.	S.S.	M.S.	Z
Between trials	5	0·0193	0·0038	..
Within trials	54	0·1351	0·0025	0·209
Total .	59	0·1544	0·0026	..

The value of Z, being non-significant, shows that the whole set of 60 observations is of homogenous composition with a total variance of 0·0026. The same result was obtained in case of sample No. 1.

It is, therefore, established that the method gave similar results with both trials. Slight fluctuations in readings may be partly attributed to atmospheric variations and partly to the fibre characteristic variation. The experiment was conducted under room temperature, facilities for conditioning the samples being not available.

(*b*) *Trials by different observers.* The question next arises whether or not the method can be reliably used by other workers. This point was also investigated in the manner noted below.

A small portion, representative of the bulk, was further separated from sample No. 2 referred to above. This was divided into five lots each consisting of approximately 10-20 fibres. It is not difficult to judge the number of fibres in small samples by experience. From each lot, a set of ten fibres was drawn out at random and the five sets so obtained were allotted to five different observers, three of them being research workers including one mathematician and two laboratory boys. They were asked to break the fibres independently and note down the extension results.

Tables IV-V show the results and their Analysis of variance :—

TABLE IV

Extension per unit inch

(Obtained by different observers)

Fibre No.	Observers					Total
	1	2	3	4	5	
1	0·43	0·31	0·47	0·45	0·40	2·06
2	0·43	0·31	0·45	0·40	0·38	1·97
3	0·45	0·38	0·40	0·39	0·40	2·02
4	0·37	0·36	0·33	0·42	0·38	1·86
5	0·45	0·39	0·41	0·40	0·40	2·05
6	0·45	0·45	0·45	0·43	0·23	2·01
7	0·41	0·38	0·30	0·40	0·33	1·82
8	0·40	0·39	0·42	0·40	0·45	2·06
9	0·36	0·51	0·45	0·40	0·40	2·12
10	0·20	0·40	0·34	0·41	0·37	1·72
Total	3·95	3·88	4·02	4·10	3·74	19·69

TABLE V

Analysis of variance

Source	D.F.	S.S.	M.S.	Z
Between observers	4	0·0075	0·00187	..
Within observers	45	0·1493	0·00331	0·285
Total	49	0·1568	0·0032	..

The value of Z obtained by this analysis is not significant. There is thus no evidence to conclude that the data are inconsistent and the observers obtained significantly different results. In other words the different workers can also operate at the apparatus with uniform results.

SUMMARY

A subsidiary contrivance has been devised and added to Oneill Hair Tester. Besides strength, the apparatus can now be used for the measurement of extension of the wool fibres.

The contrivance described is simple and records extension on a magnified scale.

It gives consistent results which are independent of personal errors.

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ABSTRACTS

A Case of Brucellosis (Aboratus fever) M. S. H. Mody (1945)
Ind. Med. Gaz. LXXXI (4-5)

A Brucellosis infection is described in a 45 years old patient at Poona. Agglutination titre was 1 in 200 on one occasion and when repeated a fortnight later it was 1 in 500. Illness lasted for 17 weeks. Although the author is aware that infection is generally contracted from bovines, he does not state the source of infection in this particular case.

H. K. L.]

A Case of Brucellosis (Aboratus fever) R. Bhuyan and R. C. Barua (1947)
Ind. Med. Gaz. LXXXII, 24

An Infection of 10½-year old Punjabee girl, who had come to Assam after spending a three months' holiday in the Punjab, is described. The patient's serum had a titre of 1 : 5000 to *Brucella* and the organism was recovered on culture. The W. B. C. and R. B. C. counts were 3,600 and 4,460,000 per c.m.m. respectively. The chief symptoms were undulant fever, painful swelling of left wrist joint, headache and poor appetite. Speculative treatment before diagnosis with injections of urea stibamine and Penicillin were of no avail; T. A. B. shocks (4 injections at intervals of four days) and pentavalent antimony preparation after the diagnosis were also found to be ineffective. After 15 weeks of illness, natural recovery occurred. The authors consider the case to be an imported one, as aboratus fever is unknown as an indigenous disease in Assam.

(Brucellosis in organized farms in Assam has been found to the extent of 20 per cent. of the tested cattle and goats associated with an abortion rate of 2 to 6 per cent.) [H. K. L.]

REVIEW

A preliminary Review of the use of penicillin in Veterinary Practice

By Imperial Chemical (pharmaceuticals) Ltd., Alderly Edge, Manchester, August 1946. pp 32

A review of the experience gained date of Penicillin in veterinary practice has been published in a booklet by Imperial Chemical (Pharmaceuticals) Ltd., Alderley Edge, Manchester for issue to veterinary surgeons. The information has been obtained from published and private reports from America, Canada, Australia and other countries. It contains a list of 83 references.

MGIPC—M—UH-1-11—18-6-48—520.

ORIGINAL ARTICLES

INCIDENCE OF BRUCELLOSIS IN DAIRY HERDS IN BENGAL

By RAM NARAIN MOHAN, Veterinary Investigation Officer, Bengal

(Received for publication on 5 July 1945)*

AS in other Provinces, attention to contagious abortions in cattle in Bengal followed the inception in 1932 of the scheme for the appointment of a Veterinary Investigation Officer, with the help of funds from the Indian Council of Agricultural Research. The observations made have been recorded in the various annual reports of the officers, but it has been considered advisable to present the results in a consolidated form, especially because Polding's [1943] publication dealing with incidence of Brucellosis in India does not cover Bengal and Assam. Moreover, though the broader features of the abortion disease in organized herds in Bengal have been found similar to those noted elsewhere, some of the observations have been quite interesting and instructive.

METHODS

In the earlier period, from 1934 to 1941, the investigations were restricted chiefly to one or two important herds, at least one of which suffered a serious outbreak of abortions and still-births. Specimens of blood serum taken from aborting and other animals of these herds, were, from time to time, and often repeatedly, despatched to the Indian Veterinary Research Institute, Mukteswar, where they were subjected to the standard tube agglutination test. Later on, from 1942 to 1945, when periodical consignments of suitable antigen and a portable outfit for a quick slide agglutination test were received from Mukteswar, the survey was extended to some more herds located in different parts of the Province. Unpreserved specimens of blood serum, generally from all available adult stock and sometimes including a few grown-up calves, were tested on the spot within a few to 24 hours, taking the usual precautions. The reactions obtained were mostly clear-cut and the percentage of doubtful reactions was small.

While some consignments of antigen went bad comparatively soon, others remained well and discriminatingly agglutinable (checked against fresher consignments) for long periods, in one case for more than a year at room temperature.

The occurrence of positive reactions in any herd was regarded rather as an indication of the presence of infection in the herd than a correct and dependable index of the extent of infection. The herd owners were advised accordingly and, where necessary, suitable hygenic control measures were prescribed.

RESULTS

The main results are summarized in Table I. Brief notes concerning individual herds and history of abortions, etc. in them are also given. It will be seen that out of thirteen herds tested, *Brucella* infection was definitely demonstrated in only eight. Herd No. 1, originally composed of Welsh and Sahiwal cattle and believed to have been previously free from infection, suffered a serious outbreak of the disease following the introduction of a batch of Jersey cattle from Australia in February 1934. The following figures give an idea about the spread of the disease in this herd.

Year	Average number of cows in the herd	Abortions	Percentage
1934-35	75 (18 Jersey)	7 (All in original herd)	9·3
1935-36	84 (do.)	30 (3 Jersey)	35·7
1936-37	98 (25 Jersey)	19 (6 Jersey)	19·4
1937-38	100	11 (9 Jersey)	11·0

*Resubmitted for publication on 28 July 1947.

Evidently, the infection was introduced along with the imported animals, spread rapidly among the original (more susceptible) stock and later also among the imported stock. This serves as a typical example of the dangers to which a previously uninfected herd is exposed when indiscriminate additions are made.

Among the herds tested, there were two buffalo herds. Like their counterpart cattle herds, and in keeping with the history of abortions in them, one was found free from infection and in the other, out of 45 animals tested, there were fourteen positive and three doubtful reactors.

Discussion

It was originally planned to test a large number of herds, but it was soon realized that that was neither practicable, since some herd owners could not be persuaded to the test, nor necessary, because in many herds abortions were reported to be either absolutely unknown over long spans of years or too few and far between to warrant a suspicion of Brucellosis. A majority of the herds left out was small, well isolated and practically self-contained units, in contrast with many of the larger herds in which abortions have been occurring frequently and to which indiscriminate additions have been made from time to time. Thus, while actual testing of a few herds of the former class has shown them to be free from demonstrable infection, even in the face of a few sporadic abortions, a varying incidence of positive reactions has been encountered in many herds of the latter class. As expected, the highest percentage of infection was in a *pinjrapole* herd which is constituted on lines welcoming all sorts of animals at all times.

Table I

Result of agglutination tests

Herd	Locality	Breed	Date of Test	Number tested	Reactions. Positive	Reactions. Doubtful	Negative	History of abortion, etc., and other remarks
1	Darjeeling Dist.	Welsh, Sahiwal and Jersey	1934-35	7	2	..	5	Herd reported free from abortions till 1933. Figures of abortions during 1934-1939 are given in text. Enquiry in 1944 revealed practically no abortions after 1940.
			1935-36	99	50	2	47	
			1936-37	32	5	..	27	
			1937-38	92	46	..	46	
			1938-39	67	35	..	32	
2	Ditto	Mixed foreign and Indian	1936-37	15	..	..	15	Herd reported to have remained generally free from abortions, but in 1936 four cows aborted within the space of five months.
			1937-38	53	6	..	47	
3	Ditto	Ayreshire and Jersey	1937-38	13	1	..	12	Absolutely no abortions for at least ten years before test and since then. The single reaction was probably non-specific.
4	Midnapore	Mainly Hariana	1941-42	12	..	3	9	No abortions until mid-May 1940, when seven cows aborted within a few months; four died soon after abortion. Malpractice was suspected.
			1941-42	15	..	5	10	
5	Dist. 24-Parganas	Buffalo	1942-43	3	2	1	..	A *pinjrapole* herd. Abortions etc. have been quite frequent all along. Herd No. 12 is the counterpart cattle herd of this buffalo herd.
			1942-43	45	14	3	28	
6	Dist. Khulna	Mainly Hariana	17-4-43	23	4	..	19	Herd started in 1938. A few infrequent abortions.
7	Barisal	Ditto	9-5-43	22	1	..	21	Abortions rare; a few about six years before test.
8	Dacca	Hariana	7-9-43	54	..	..	54	Abortions very rare. Occasional retained placenta.

Herd	Locality	Breed	Date of Test	Number tested	Reactions.			History of abortion, etc., and other remarks
					Positive	Doubtful	Negative	
9	Dacca	Buffalo	8-9-43	17	..	..	17	Practically no abortions.
10	24-Parganas	Mainly Hariana	19-11-43	25	..	..	25	Abortions very rare; one just before test.
11	Dacca	Ditto	9-1-44	31	..	2	29	Abortions practically unknown until a couple of cows calved prematurely and one aborted some time before test.
12	Dist. 24-Parganas	Mixed Indian	25-2-44	49	18	3	28	Same as in Herd No. 5.
13	Ditto	Sahiwal, Hariana, Tharparkar	24-8-44 25-8-44 30-8-44	65	10	2	53	A few infrequent abortions and allied troubles. The three bulls tested proved negative.
				Total 739	194	21	524	

SUMMARY

Results of 739 agglutination tests for Brucellosis conducted in 13 dairy herds in Bengalare presented. *Brucella* infection was definitely established in only eight herds. One of the two buffalo herds examined was found positive with over 30 per cent reactors. The results corresponded well with history of abortions, etc. While the incidence of abortions and percentage of reactors were comparatively high in bigger commercial and other herds welcoming frequent additions, these were low or *nil* in many smaller self-contained herds. Majority of the herds in Bengal belong to the latter class.

ACKNOWLEDGEMENTS

Herds Nos. 1, 2 and 3 were tested at Mukteswar through blood serum sent by my predecessors Messrs M. B. Menon and Balwant Singh. The paper includes some records left by them as well as some information kindly supplied by Mr. J. B. Polding, formerly Research Officer, Contagious Abortion, Mukteswar, with regard to Herd No. 4, which was tested by him when he visited Bengal.

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THE RELATIVE RATE OF ABSORPTION OF DIFFERENT OILS AND FATS

By V. R. Bhalerao, D. Venkatappiah and C. P. Anantakrishnan, Imperial Dairy Research Institute, Bangalore

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THE relative nutritive value of natural oils and fats is generally judged by the rate of assimilation and the percentage of utilization in the body. With regard to the rate of absorption of different fats, a number of investigations have been reported. Steenbock *et al* [1936] determined the comparative rate of absorption of different fats in experiments with rats following the technique of Irwin *et al* [1936] and indicated that in general lard and hydrogenated oils were absorbed at approximately the same rate, while butter oil, halibut liver oil and codliver oil were absorbed at a more rapid rate. Irwin *et al* [1936] found that hydrogenation decreased the rate of absorption of an oil, but the variations in the melting point below body temperature were without effect. Basu and Nath [1946] found that there existed a real difference in the rate of absorption of cow butter fat, mustard oil, cocoanut oil, sesame oil and groundnut oil in a four hour period, but after six hours all the fats were absorbed almost to the same extent.

The present communication deals with the rate of absorption of the fats commonly occurring in India namely of butter fats of cow, buffalo, goat and sheep of edible oils ; sesame, groundnut ; cocoanut, safflower, mustard and cotton seed, of *vanaspathies* from groundnut, sesame and cottonseed oils and of the body fats from cow, buffalo, goat and sheep.

Experimental

The technique of Irwin *et al* [1936] was adopted. Adult rats from 4 to 7 months old weighing about 200 gm. were fasted for 48 hours, during which period water was given *ad libitum*. With the help of the stomach tube, 1·5 c.c. of melted fat was then delivered into the stomach. In a similar way 1·5 c.c. of the fat was delivered into beaker and weighed. The average of three such weighings was taken as the weight of the fat fed.

At the end of four hours after feeding the fat, rats were decapitated and the abdominal cavity opened. The stomach, intestine and caecum were removed immediately from the body cavity. Each section was filled with distilled water and after ten minutes the contents were emptied in a beaker. The sections were then filled with petroleum ether (40°C.—60°C.) and after ten minutes the contents were emptied in the same beaker. The stomach and the caecum were cut wide open and washed thoroughly with a jet of water and petroleum ether. The intestines were stripped manually. The contents of the beaker were exhaustively extracted with petroleum ether, the ether solution was dehydrated and the solvent removed in a tared dish and the fat residue dried at 100°C. for three hours and weighed.

Ten rats were used for each sample. As the sex was found not to play an important role in the rate of absorption by Irwin *et al* [1936] the rats of both sexes were used.

In calculating the absorption values, the amount of fat present in the stomach after 48 hours fasting was also taken into consideration. The rats were killed after the fasting period and the fat extracted as before.

Results

The rates of absorption together with their standard errors of the mean for different fats are shown in Table I.

It can be observed from Table I that the rates of absorption of the milk fats form the herbivorous animals of Indian origin range from 40·3 to 48·4 per cent in four hour period. The percentage of absorption of cow and buffalo *ghee* is almost similar while goat *ghee* shows a slightly higher absorption. The body fats showed a significantly lower rate of absorption as compared with cow *ghee*.

It was found that at the end of four hours the stomach of the animals fed body fats contained a visible hard fat. This lower rate of absorption is probably due to the high melting point of the fat as seen from Table II.

TABLE I

The Rate of Absorption of Oils and Fats in 4 hours Digestion Period

No.	Name of Fat	Absorption (per cent)	Standard error of the mean	't' for significance (All fats compared to Cow Ghee)
1	Cow Ghee. M. P. 36·5°C.	45·7	±2·58	..
2	Buffalo Ghee. M. P. 36·3°C.	45·6	±2·48	0·02
3	Sheep Ghee. M. P. 35·4°C.	40·3	±1·60	1·36
4	Goat Ghee. M. P. 34·3°C.	48·4	±1·41	0·88
5	Cow body fat. M. P. 49·6°C.	31·3	±0·77	5·10
6	Buffalo body fat. M. P. 52·3°C.	33·6	±1·33	3·91
7	Sheep body fat. M. P. 49·6°C.	30·5	±1·41	4·97
8	Goat body fat. M. P. 49·4°C.	32·0	±1·00	4·67
9	Groundnut Oil.	39·4	±0·94	2·20
10	Sesame Oil	45·1	±2·05	0·18
11	Safflower Oil	41·2	±1·72	1·40
12	Cocoanut Oil M. P. 27·6°C.	45·7	±2·49	0·00
13	Mustard Oil	27·1	±1·56	5·94
14	Cotton seed Oil	38·9	±7·51	1·84
15	Groundnut V. M. P. 34·1°C.	36·5	±1·14	2·99
16	Groundnut V. M. P. 37·0°C.	34·8	±1·96	3·26
17	Groundnut V. M. P. 39·0°C.	33·6	±0·88	4·23
18	Sesame V. M. P. 37·1°C.	34·8	±1·53	3·51
19	Sesame V. M. P. 38·7°C.	32·5	±1·05	4·56
20	Sesame V. M. P. 39·3°C.	30·7	±1·20	5·09
21	Cotton seed V. M. P. 35·5°C.	36·2	±3·57	2·14
22	Cotton seed V. M. P. 37·3°C.	34·8	±1·79	3·37
23	Cotton seed V. M. P. 38·5°C.	30·9	±1·74	4·42

t= for significance = 2·262.

With regard to edible oils, mustard oil is found to be least absorbed while sesame and cocoanut oils happened to be absorbed to the same extent as cow *ghee*. The other oils, safflower, groundnut and cotton seed, though they were absorbed to a lesser degree than cow *ghee*, did not show any significant difference.

Vanaspathies, in general, are found to be less absorbed. If the rates of absorption of groundnut *vanaspathies* are taken into consideration, it is seen that as the melting point of the sample increases from 34·1°C. to 39·0°C, the absorption correspondingly decreases from 36·5 per cent to 33·6 per cent Though the sample having a melting point of 34·1°C. compared favourably well with groundnut oil, the rates of abosrption of other two *vanaspathies* were significantly lower. The rates of absorption of the sesame as well as cotton seed *vanaspaties* showed the same trend, i.e., higher the melting point, the lower was the rate of absorption.

This discrepancy in the rates of absorption of different fats can be partly explained by following closely the changes that take place in a fat as digestion proceeds. This has been done by analyzing the intestinal residues from each group for B. R. Index, saponification value and the iodine values and the data are shown in Table II. A glance at Table II reveals clearly that there is an increase in the B. R. Index and a lowering of the iodine value in the residual fat.

TABLE II

Chemical Characteristics of the fats fed and the undigested fat remaining in the stomach

Sample No.	Fat used	Melting point °C	B. R. Reading at 40°C.		Iodine value		Saponification value.		Reichert Meissel value.	
			Original	Undigested	Original	Undigested	Original	Undigested	Original	Undigested
1	Cow Butter fat	36·5	43·5	47·8	36·5	31·5	220·6	200·5	25·7	18·2
2	Buffalo Butter fat	36·3	42·3	47·5	32·8	30·7	224·6	205·0	29·6	17·8
3	Goat Butter fat	34·3	40·7	47·4	27·2	23·7	231·0	228·7	25·3	18·0
4	Sheep Butter fat	35·4	45·2	51·5	35·9	32·1	221·8	214·6	25·0	18·8
5	Cow body fat	49·6	45·4	48·3	29·7	27·7	203·6	192·4	..	..
6	Buffalo body fat	52·3	44·4	49·4	31·2	24·1	191·6	191·2	..	..
7	Sheep body fat	49·6	46·2	54·6	36·1	32·0	197·5	191·6	..	..
8	Goat body fat	49·4	46·3	51·7	32·4	25·3	198·9	198·7	..	..
9	Cocoanut oil	27·6	35·6	39·8	8·2	12·1	259·0	231·4	..	..
10	Sesame oil	..	59·3	69·9	101·9	70·9	190·6	184·9	..	..
11	Safflower oil	..	60·5	72·3	112·8	69·0	192·0	184·0	..	..
12	Groundnut oil	..	56·2	61·8	87·5	67·3	190·5	187·2	..	..
13	Cotton seed oil	..	60·0	68·6	99·3	59·1	191·6	188·0	..	..
14	Mustard oil	..	60·2	68·8	99·2	60·4	170·9	197·0	..	..
15	Groundnut Vanaspathi	34·1	54·6	59·6	78·7	59·1	199·0	191·7	..	..
16	Ditto	37·0	52·9	59·8	73·5	57·7	198·2	187·4	..	..
17	Ditto	39·0	51·7	55·8	69·8	56·5	197·4	189·9	..	..
18	Sesame Vanaspati	37·1	50·6	55·9	68·4	65·1	191·3	186·2	..	..
19	Ditto	38·7	50·4	55·1	65·3	60·2	189·6	183·9	..	..
20	Ditto	39·3	50·0	54·9	62·6	50·7	187·5	185·9	..	..
21	Cottonseed vanaspathi	35·5	52·3	56·1	63·8	54·9	193·7	194·0	..	..
22	Ditto	37·3	51·1	54·4	68·1	66·2	189·3	187·4	..	..
23	Ditto	38·5	51·0	53·3	67·8	66·9	190·9	179·5	..	..

It is also seen from Table II, that there is a lowering in the saponification value and Reichert-Meissel value of the butter fats. Though the differences are not large, there is an indication that the short chain fatty acids are more readily absorbed than the long chain fatty acids. There is not so much lowering in the iodine value as in the case of oils or *vanaspathies* indicating preferential absorption in the gastro-intestinal tract of the short chain acids as compared to the unsaturated acids in case of butter fats. The presence of lower fatty acids in the butter fats is a probable explanation of their quicker absorption as compared to the other fats. Cocoanut oil also behaves in the same way as butter fats, as is seen from the fact that the saponification value of the residual fat is lowered considerably from 259·0 to 231·4 indicating a quicker absorption of the lower acids. In case of the body fats there is no definite indication except the fact that the unsaturated acids are absorbed proportionately more as evident from the slight lowering of the iodine value of the undigested fat. There is a marked change in the nature of edible oils during the digestion. The iodine value is almost reduced to two-thirds in all the oils except cocoanut oil. This leads to the conclusion that the unsaturated acids, namely oleic and linoleic, are more readily digested than the saturated acids.

The lowering of absorption of the oils by hydrogenation may be explained by chemical changes that are brought about during the process. The linoleic acid is converted to oleic and iso-oleic acid and finally into stearic acid, thus the proportion of oleic and linoleic acids gradually decrease with a proportionate increase in the stearic acid content. This lowering of the unsaturated acid contents in the fat is probably responsible for the lowering of its rate of absorption.

It is thus seen from the results that the short chain fatty acids and the unsaturated acids, particularly oleic and linoleic acids, are more readily absorbed than the long chain saturated fatty acids in four hours absorption period.

Summary

The realtive rate of absorption of cow, buffalo, goat and sheep butter fats, the body fats of cow, buffalo, goat and sheep, the vegetable oils, cocoanut, sesame, groundnut, cottonseed, safflower and mustard, the hydrogenated groundnut oils of melting points of 34·1°C., 37·0°C. and 39·0°C., the hydrogenated sesame oils of melting points of 37·1°C., 38·7°C., 39·3°C. and the hydrogenated cottonseed oils of melting points of 35·5°C., 37·3°C. and 38·5°C. were determined after four hours by feeding 1·5 c.c. of each fat. The undigested fats in the alimentary canal were also examined for their chemical characteristics.

It was found that sesame oil and cocoanut oils were absorbed at the same rate as cow butter fat, while sheep butter fat, safflower oil, groundnut oil and cottonseed oil were absorbed somewhat more slowly, but the absorption was not significantly less than that of cow *ghee*. All the body fats and hydrogenated oils were significantly less absorbed as compared with cow *ghee*. It was also found that hydrogenation lowered the rate of absorption of an oil.

It is suggested that the short chain fatty acids are more readily absorbed than the long chain fatty acids and among the long chain fatty acids, the unsaturated acids are more readily absorbed than the saturated acids.

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RESAZURIN TEST FOR ASSESSING THE STERILITY OF DAIRY UTENSILS

By F. D. Aibara and H. Laxminarayana, Indian Dairy Research Institute, Bangalore

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IT is well known that dairy utensils and milk plant, which are not thoroughly cleaned and efficiently sterilized, represent the most important sources of bacterial contamination in milk. Accordingly the efficiency of the methods of cleansing and sterilization in a dairy is required to be checked periodically by testing the equipment and utensils for sterility. For this purpose the plate counts of either the rinsings or of swabs are most widely employed. Since absolute sterility, i.e. complete freedom from all living organisms, is impracticable under commercial conditions, maximum limits of plate counts are allowed for different types of utensils varying from 10 per ml. of rinse solution in the case of bottles to 100 per ml. for milk pails and 500 per ml. for churns [Chalmers, 1945]. An official technique for the examination of washed churns and suitable limits of plate counts for evaluating the efficiency of farm sterilization methods have also been prescribed by the British Ministry of Agriculture [1943]. The plate count method is, however, complicated and involves considerable delay in obtaining results. The presumptive coliform test which has also been used for the purpose is not found to be of much value, particularly in the case of bottles [Mattick and Hoy, 1937; Barkworth, 1941].

Thomas [1943] suggested the use of resazurin as a simpler and quicker test for detecting churn sterility. The method was subsequently modified by Davis and Watson [1943], according to whom the rinse solution from the churn is added to sterile separated milk and incubated at 22°C. for 24 hours. The incubated sample is then subjected to the resazurin test and if the colour is not reduced to a lilac shade (disc 5) in half an hour, it is an indication of a satisfactory degree of sterility in the churn. Discs 5 to 3½ are taken to denote a 'doubtful' condition while reduction to disc 3 or below is an index of unsatisfactory condition. A good agreement between these resazurin standards and the plate count limits laid down by the Ministry of Agriculture was found by Davis and Watson. The application of this test for examining the common dairy utensils used for handling milk in this country would be of great practical value in improving the bacteriological quality of market milk. Some modifications in the technique and interpretation of the test may, however, be called for in view of the different climatic and environmental conditions prevailing here. The present study was carried out with the object of examining the suitability and reliability of the resazurin test in comparison with plate counts for determining the sterility of bottles, milk pails, churns, receiving tank and cooler.

Experimental

The tests were carried out on milk bottles (½ lb. and 1 lb.), milk pails (10 lb.), churns (100 lb.) receiving tank and cooler (surface type) after cleaning and sterilizing them in the following manner so as to produce in them degrees of sterility ordinarily found in utensils handled in commercial dairies.

Bottles (½ lb. and 1 lb.)	*I Series*	Washed with washing soda and soapnut powder rinsed with hot water (70°C.) and dried in air for 4-5 hours by holding them mouth downwards in a slanting position.
	II Series	Washed as above and then sterilized in hot air oven (160°-170°C.) for one hour, two hours and four hours respectively using three separate batches for each treatment.
Pails	*I Series*	Washed as above.
	I I Series	Washed and steamed for two minutes with a steam jet and dried.
Churns	....	Washed as above and steamed for five minutes with a steam jet and dried.
Receiving tank and cooler	....	Washed and steamed thoroughly in all parts by means of a hose pipe and dried.

The washed or sterilized utensils as mentioned below were thoroughly rinsed with quarter-strength Ringer's solution in quantities indicated against each.

Half pound bottles	10 ml.
One pound bottles	20 ml.
Milk pails	150 ml.
Churns	500 ml.
Receiving tank	500 ml.
Cooler	500 ml.

In the case of the cooler, the whole surface was thoroughly scrubbed with a sterile brush using 500 ml. solution for the purpose. The utmost precautions were observed so as to carry out the operations under aseptic conditions and absolutely sterile apparatus was used for performing the tests. Slight exposure to outside contamination could not, however, be avoided in the case of the receiving tank and cooler. In all the trials controls were employed and wherever extraneous contamination was suspected, the results of such experiments were discarded.

Plate counts. Portions of the rinsings (1 ml. and 3 ml. in the case of sterile utensils and suitable dilutions in other cases) were plated in triplicate on milk agar. The plates were incubated at 37°C. for 48 hours and averages of the colony counts taken excluding those showing more than 20 per cent discrepancies.

Presumptive coliform test. Portions of the rinsings in appropriate serial dilutions were tested for the presence of coliform bacteria in McConkey broth tubes (duplicate) after 48 hours' incubation. Negative tubes were incubated for another 48 hours and the reactions noted.

Resazurin test. One ml. portion of the rinse solution was added to 10 ml. sterile separated milk (aged for not less than two weeks) and then incubated at 22°C. for 24 hours. The incubated sample was then thoroughly shaken, one ml. of 0·005 per cent resazurin solution added and the resazurin colour changes at 37°C. after 10, 20, 30, 60, 120 and 180 minutes observed using the standard Comparator for comparing the colours [Davis and Watson, loc. cit.].

In the course of the preliminary trials with bottles, it was found that the incubated samples in respect of both the washed and partially sterilized series, invariably showed a rapid resazurin reduction (pink or colourless) in 30 minutes. This was probably due to the long incubation of 24 hours allowing an undue development of the initial flora. Furthermore, any substantial shortening of this period would help in considerable saving of time in obtaining the results. Accordingly other time-temperature combinations for incubating the rinse samples were tried out. Of these, incubation for 16 hours at 22°C. was found to give most satisfactory results and was also convenient as it corresponded with the farm routine. Hence the resazurin test was carried out on rinse samples incubated for 16 hours at 22°C.

Results

The mean colony counts (per ml. of rinse solution) obtained in respect of different utensils and the corresponding resazurin reduction stages at the end of 30 minutes, one hour and two hours are given in Table I. The resazurin colour shades (blue, lilac, mauve, pink mauve, purple pink, pink and white) are expressed in terms of disc numbers 6 to 0 (indicated on the resazurin comparator) for facilitating interpretation. There was no appreciable colour change at the end of 10 minutes due to the interval being too short and the reduction after three hours had invariably advanced to either a pink or colourless stage in almost all cases. Hence these observations are not included in the data. The results of the coliform test for each group of samples are also given.

It is seen that the resazurin colour changes (disc numbers) are fairly comparable with plate counts in bringing out the marked difference between washed and sterilized utensils in their standards of cleanliness. There is, however, some discrepancy observed in the case of one lb. bottles since the washed series also show high disc numbers comparable to those of the sterilized ones. By increasing the period of sterilization from one hour to two and four hours slightly higher disc numbers are obtained. The plate counts for all the groups of sterilized bottles, with the exception of half pound bottles subjected to sterilization for one hour, are below 10 per ml. of rinse solution [satisfactory standard according to Chalmers, 1945] and the corresponding resazurin disc numbers are above

5, 5 and 3 at the end of 30 minutes, 1 hour and 2 hours respectively. In the case of milk pails and churns (subjected to steaming) the plate counts are below 100 and 500 per ml. respectively ['satisfactory' — Chalmers, 1945], while resazurin is reduced to disc 5, 4 and 2 respectively in the above intervals. The receiving tank and cooler have registered very high counts with resazurin reduction going up to a colourless stage even at 30 minutes. The latter phenomenon is obviously due to the presence of large numbers of actively reducing types (mainly lactic streptococci and micrococci) in these utensils which are not easily eliminated. The application of the resazurin test in the present form does not, therefore, provide any useful information in the case of the receiving tank and cooler. The coliform test has given uniformly negative results (except for the last two categories) and does not appear to be of much value in judging the efficiency of sterilization [Barkworth, loc. cit.].

TABLE I

Comparison of plate count, resazurin reduction and coliform test for different utensils

Type of utensil and treatment given	Number of samples	Plate count per ml. of rinse (Geo. Mean)	Mean resazurin disc number for incubated samples at the end of			Presumptive coliform test
			30 minutes	1 hour	2 hours	
Bottles (8-10 oz.)—						
Washed only	11	358	$3\frac{1}{2}$	$2\frac{1}{2}$	$1\frac{1}{2}$	1 ml.
Sterilized for one hour	4	12	$5\frac{1}{4}$	5	$3\frac{3}{4}$	1 ml.
Sterilized for two hours	4	5	6	6	$5\frac{1}{4}$	1 ml.
Sterilized for four hours	4	6	6	$5\frac{3}{4}$	5	1 ml.
Bottles (1 lb.)—						
Washed only	12	69	$5\frac{1}{2}$	$4\frac{1}{2}$	3	1 ml.
Sterilized for one hour	4	3	$5\frac{1}{2}$	$4\frac{1}{2}$	3	1 ml.
Sterilized for two hours	4	6	6	$5\frac{3}{4}$	$4\frac{1}{4}$	1 ml.
Sterilized for four hours	4	6	$5\frac{1}{2}$	$4\frac{3}{4}$	$3\frac{1}{2}$	1 ml.
Milk pails—						
Washed only	10	5,479	$2\frac{3}{4}$	2	$1\frac{1}{2}$	10 ml.
Steamed for 2 minutes	11	7	5	4	2	10 ml.
Churns—						
Steamed for 5 minutes	9	431	5	$4\frac{1}{2}$	$2\frac{3}{4}$	10 ml. (pos. in)
Receiving tank—						
Steamed for 5 minutes	9	194,200	$\frac{1}{4}$	0	0	1 ml.
Cooler—	5	194,700	0	0	0	1 ml.

On the basis of the above data resazurin reduction up to disc 5 (lilac stage) in 30 minutes may be taken to indicate a satisfactory degree of sterility in the case of pails and churns while a higher resazurin standard (say disc $5\frac{1}{2}$) can be obtained in regard to bottles. It is also clear that there is no advantage in observing resazurin colour changes beyond 30 minutes. The discrepancies shown between bottles and other utensils may be ascribed to the higher limits of bacterial counts allowed for the latter and also the different types of flora surviving in the utensils after the washing and sterilization treatments. In the case of bottles the predominant organisms were found to be staphylococci and micrococci, while lactic streptococci were most prominent in rinse solutions from pails and churns. The latter organisms are known to grow faster in milk and also reduce resazurin more actively [Jones and Davis, 1944]. The British workers [Davis and Watson, loc. cit.] have suggested that reduction of resazurin to stages higher than disc 5 (lilac) should be regarded as indicating satisfactory churns. Considering the differences between the two countries in respect of climatic conditions and nature of bacterial flora in milk, the slightly lower standard of disc 5 indicated in the present study appears to be suitable. The same standard can also be adopted in the case of bottles without giving rise to any serious errors of judgement because in actual practice it would be difficult to distinguish between discs $5\frac{1}{2}$ and 5 while the development of a slight mauve colour (lower than disc 5) is more clearly detected.

The comparative efficiency of the resazurin test in detecting inefficiently sterilized utensils (taking reduction up to disc 5 or lilac stage as a criterion of satisfactory condition) is further examined in Table II.

TABLE II

Grading of utensils on plate count and resazurin test

(Figures in the Table represent number of samples distributed on the two scales)

Name of utensil	Plate count grade	Number of samples	Classification on resazurin scale	
			Satisfactory (Disc 5 and higher)	Unsatisfactory (Below disc 5)
Bottles	Satisfactory (be ow 10 per ml.)	13	12	1
	Unsatisfactory	18	8	10
Pails	Satisfactory (below 100 per ml.)	6	5	1
	Unsatisfactory	12	2	10
Churns	Satisfactory (below 500 per ml.)	6	5	1
	Unsatisfactory	2	2	..

Out of 25 utensils satisfying the plate count standard, 22 are passed by the resazurin test and of 32 utensils failing on the former scale only 20 are condemned by the latter test. On the other hand 20 out of 23 utensils failing the resazurin test are also condemned on the plate count standard. Thus, the resazurin test, although capable of detecting most of the efficiently or inefficiently sterilized vessels, fails to pick out an appreciable number of samples condemned on the plate count. This anomaly is easily explained considering that (*a*) the slightest contamination occurring at the time of plating and other inevitable sources of error in the technique will give rise to a higher count without affecting the reduction test to the same degree, and (*b*) the flora surviving inefficient sterilization in some cases (e.g. bottles) may be of an inert type without much influence on the reduction of resazurin. Accordingly the resazurin test would appear to be less sensitive and accurate than plate count in detecting faulty methods, while it is more reliable in indicating the presence of actively reducing types of organisms that have survived in the containers and which are apt to bring down the keeping quality of milk. In any case failure of the utensils to pass the resazurin standard is a definite indication of inefficient methods and this factor is of sufficient practical value in controlling the operations under commercial conditions.

It is possible that many samples condemned by the resazurin test may come under the ' doubtful ' class suggested by Davis and Watson [loc. cit.]. The present data have not provided any basis for fixing up an intermediate standard. If the object of applying the test is to determine whether the method of washing and sterilization of utensils is effective enough or not, the provision of any such intermediate grade has no practical significance. Further application of the test under actual commercial conditions is necessary for elucidating the above point.

SUMMARY

The applicability of the resazurin test for assessing the sterility of dairy utensils, e.g. bottles, milk pails, churns, receiving tank and cooler, as a means of checking the efficiency of the cleansing and sterilization methods in the dairy has been examined.

2. The resazurin test was carried out on rinsings (1 ml. mixed with 10 ml. sterile separated milk and then incubated at 22°C. for 16 hours) and the colour changes in half an hour (at 37°C.) fairly agreed with the plate counts of the rinsings. Resazurin reduction to a stage not lower than disc 5 (lilac shade) was an indication of satisfactory quality in all the utensils.

3. The anomalies found between different utensils and between plate counts and resazurin reduction have been discussed and the practical utility of the dye reduction test for application under commercial conditions indicated.

4. The resazurin test in the present form has not been found useful for examining receiving tank and cooler.

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A COMPARATIVE STUDY IN THE USE OF DIFFERENT MEDIA FOR THE ESTIMATION OF COLIFORM ORGANISMS IN MILK

By M. A. Krishnaswamy and H. Laxminarayana, Indian Dairy Research Institute, Bangalore

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In view of the great importance attached to the presence of members of the coli-aerogenes group in milk and milk products, the attention of workers in the field has been focussed on the development of suitable methods for their detection and estimation. The dilution extinction method (originally introduced by Lister) using various liquid media has been widely employed for the purpose and recently plating on solid media for the enumeration of coliform organisms and the direct differentiation of *coli* and *aerogenes* types have also come into practice. Most of the media were originally introduced for water analysis and subsequently suggested for the examination of milk.

MacConkey's lactose peptone bile broth [1905 and 1908] has been almost exclusively used for the presumptive coliform test in England [Ministry of Agriculture, 1934] while in the United States a standard lactose broth, differing from the former mainly in the omission of bile salt, was in general use for a long time. The serious drawbacks of the latter medium in permitting the growth of other types of organisms and giving many false positives were realized early and this led to the introduction by various American workers of several selective liquid media, which, while permitting the growth of coliform bacteria, would inhibit other organisms. Two of these namely, two per cent brilliant green bile broth [Jordan, 1927] and formate ricinoleate broth [Stark and England, 1935] have been generally regarded as most satisfactory [Farrell, 1937; McCrady and Langevin, 1932; McCrady and Archambault, 1934; Noble and White, 1935; Babel and Parfitt, 1936] and they are now recommended for the presumptive coliform test in the bacteriological examination of milk in the United States [American Public Health Association, 1941].

Solid media were originally employed for differentiating between the various members of the *coli-aerogenes* group and confirmation of positive broth tubes in the presumptive test. MacConkey's agar [1905] has been most commonly employed in England for the purpose, although several other selective media namely, Eijkman's medium, Simmon's citrate agar and Koser's citrate medium, have also been used for distinguishing between the two groups [Wilson and colleagues, 1935]. In other countries, Endo's [1904] medium, litmus lactose agar, Levine's [1918] eosin methylene blue agar and recently violet red bile agar (Difco laboratories, U.S.A.) and desoxycholate agar [Liefson, 1935] have been widely used.

The employment of solid media for the enumeration of coliform organisms and direct differentiation of *coli* and *aerogenes* types have received attention only recently. Its main advocates have been Tonney and Noble [1932] who recommended a complex ferrocyanide citrate agar medium. Kon [1932-33] could not get clear-cut results on any of the solid media suggested by previous workers. Wilson *et al* [loc. cit.] conclude that MacConkey's medium (whether liquid or solid) is of great value in the estimation of coliform bacilli. According to McCrady and Archambault [loc. cit.], Bartram and Black [1937], Babel and Parfitt [loc. cit.] and Miller and Pricket [1938] violet red bile agar gives most satisfactory results in 24 hours, while Chilson and Eglinton [1936] and Yale [1937] advocate the use of desoxycholate agar. The last two media namely, violet red bile agar and desoxycholate agar, have now been recommended for the presumptive coliform test in milk and eosin methylene blue or Endo's agar for subsequent isolation of colonies for confirmation and identification [American Public Health Association, 1941].

Although no legal standards or official methods for the bacteriological examination of milk have been prescribed in India, the presumptive coliform test using MacConkey's broth [Ministry of Agriculture, 1934] is employed in certain public health and dairy laboratories for assessing the hygienic quality of water and milk. Cunningham and Raghavachari [1924] and Raghavachari [1926] studied the application of some recent methods of differentiating lactose fermenting organisms

in faeces, soil, milk and water under Indian conditions and found that MacConkey's bile salt lactose broth was the most suitable medium for the purpose. A similar conclusion has been reached by Raghavachari and Sitarama Iyer [1934, 1936 and 1939], who examined the use of several media for estimating coliform contamination in water. In his studies on the types of colon organisms present in milk supplies in Pusa, Walton [1931] used MacConkey's broth with litmus as a selective medium for the detection and growth of coliforms and Endo's agar for confirming them. The use of solid media for estimating coliform organisms in Indian milks does not seem to have received attention.

There is thus considerable divergence of opinion as to the best medium for carrying out the presumptive coliform test in milk. While the English workers favour McConkey's broth, the American authors recommended several liquid as well as solid media for the purpose. The most suitable one from among them may be selected and adopted for use in this country. It is well known that the nature of bacterial flora developing in milk is characteristic of the climatic and environmental conditions and since India differs so widely from the western countries in these respects, any bacteriological test or medium evolved there must be examined for its applicability as well as reliability in testing Indian milks before accepting it with confidence. Accordingly the present study was undertaken with a view to compare some of the well-known liquid and solid media available for the estimation of coliform organisms in milk.

Experimental

Media compared. The following media, which could be obtained locally or prepared in th laboratory from available materials, were compared for estimating the coliform organisms in milk by the dilution and plating methods :

Liquid media	*Solid media*
MacConkey's broth	MacConkey's agar
Formate ricinoleate broth	Endo's agar
Brilliant green bile broth	Eosin methylene blue agar
	Violet red bile agar

With the exception of MacConkey's broth, the media were constituted from the Difco dehydrated products (U.S.A.). The MacConkey's broth was prepared in this laboratory according to the formula given by Chalmers [1945].

In the course of the investigation, the Difco preparation of MacConkey's agar was exhausted early and further quantities could not be obtained. Some preliminary trials were, therefore, undertaken to prepare the medium in this laboratory. A medium prepared from ordinary ingredients according to the formula given by Chalmers [1945] or using Difco formula gave very unsatisfactory results. The chief trouble seems to lie with the quality of peptone, bile salt and indicators, as a result of which characteristic colonies of coliform organisms could not be obtained and other types of organisms could not be prevented from growing on the medium and giving misleading results. Ultimately the following composition, employing Difco ingredients and a suitable concentration of the indicators, was found to give satisfactory results comparable with those yielded by the dehydrated product and was adopted in subsequent work :

Bacto-peptone	17 gm.
Proteose-peptone	3 gm.
Bacto-lactose	10 gm.
Bacto-bile salts	1·5 gm.
Sodium chloride	5 gm.
Shred agar	17 gm.
Neutral red (B.D.H.) (0·5 per cent aqueous solution)	6 ml.
Crystal violet (B.D.H.) (0·1 per cent aqueous solution)	2 ml.
Tap water	1 litre.

(Final *p*H 7·1)

All the media were sterilized at 15 lb. pressure for 20 minutes and incubated at 37°C. for 3 to 4 days for detecting and weeding out any contaminated tubes before employing them for the experiments.

Presumptive test. Three successive tenfold dilutions of the milk sample (corresponding to its general level of bacteriological quality) were prepared in saline water in the usual manner, *i.e.*, by adding one ml. of milk to 9 ml. of saline water for making 10^{-1} ml. dilution and preparing further dilutions from the latter. Where the dilution series started from 10^{-2} ml., one ml. of original milk was added to 99 ml. water to make up 10^{-2} ml. dilution and subsequent dilutions made from it in the same manner. One ml. portions of each dilution were inoculated into the three broth (liquid media) tubes in triplicate and also plated in duplicate on the four solid media. After the agar had solidified in the petri dishes about 4 ml. of the same medium was poured over it in order to prevent the formation of atypical surface colonies. The broth tubes as well as the plates were incubated at 37°C. and examined after 24 hours and 48 hours in the beginning. All the liquid media which were to show the production of acid and gas did so invariably in 24 hours and there was no advantage in continuing the incubation for 48 hours. In the case of plates, the maximum number of colonies were obtained in 24 hours and further incubation only resulted in an increase of the size of the colonies and in many instances it permitted the growth of certain slow acid producers which gave misleading results. Accordingly, after making observations at the end of 24 hours, only such of the tubes and plates as gave negative or doubtful results were incubated for further 24 hours and observed. None of them, however, gave any positive reactions at the end of 48 hours. The production of acid and gas (more than 10 per cent in the Durham's tube) in at least two out of the triplicate broth tubes and the appearance on the plates of typical dark-red or brick-red colonies of at least 0·5 m.m. in diameter and not less than five (average of duplicates) in number were taken as positive presumptive evidence of the presence of coliform organisms in any particular dilution. The total number of coliform colonies on the plates was also counted.

Completed test for confirmation of the presumptive evidence. All the positive fermentation tubes as well as selective agar plates in each of the dilutions were subjected to the confirmatory test according to the procedure recommended by the American Public Health Association [1941]. In the case of positive plates showing more than five colonies any two of the typical colonies were picked up for confirmation.

The results of the above tests carried out on 53 samples of milk (incluing farm-produced milk, village milk, bulk samples handled in dairies and pasteurised milk) are given in Tables I and II.

RESULTS

A comparison of the combined results of the presumptive coliform test in different media for all the samples grouped according to the dilutions at which positive reactions were indicated is given in Table I. In order to bring out clearly the comparative efficiency of each medium in giving a clear reaction in 24 hours, in the same material, the number of samples positive or negative in MacConkey's broth at different dilutions have been separately indicated in two series. Taking them as standard, the number of corresponding samples in each group showing an identical reaction in the other media is indicated. Since all the presumptive positive tubes as well as plates were positive to the confirmatory test, no separate column is provided in Table I for illustrating this point.

It will be seen (Table I) that practically all the samples, whether showing a positive or negative presumptive test in MacConkey's broth, have given similar reactions in the other two liquid media. The few discrepancies observed may be due to sampling errors. There were no false positives encountered as the results of the presumptive test in all the media were confirmed without exception. This is in accordance with the observation of Wilson *et al* [loc. cit.] who did not experience this trouble in the case of MacConkey's broth in a large number of trials with water and milk, while Farrell [loc. cit.] mentions the occurrence of false positives in the case of brilliant green bile broth.

As regards the solid media, the number of positives recorded by them is somewhat lower than those indicated by MacConkey's broth in the corresponding dilutions. This anomaly may be due to the limit of five or more colonies taken as positive evidence being too rigorous a condition, because samples giving even one colony on the plate may show development of acid and gas in the liquid medium. In a number of cases giving a positive test in MacConkey's broth the corresponding plates have shown one, two, three or four colonies which have not been taken into account. However,

no sample showing five or more colonies on the solid media has given a negative test in MacConkey's broth and similarly almost all the samples negative in MacConky's broth have also given a negative test on the solid media.

TABLE I

Comparison of the results of presumptive coliform test in different media with those in MacConkey's broth as standard

Dilution in ml.	Number of samples positive or negative in MacConkey's broth	Number of corresponding samples giving identical results in other media					
		Formate ricinoleate broth	Brilliant green bile broth	MacConkey's agar	Endo's agar	Eosin methylene blue agar	Violet red bile agar
			(Positive series.)				
100 —1	4	3	4	1	2	1	1*
10 —2	21	21	21	19	19	20	20
10 —3	30+1*	29	29	28	27	28	27
10 —4	23	21	18+1*	14	18	16	16
10 —5	13	12	11	10	10	7	10
10 —6	8	8	8	8	8	7	8
	6	6	5	3	2	2	1
			(*Negative series*)				
100 —1	2	2	2	2	2	2	2
10 —2	7	6	7	7	7	7	6
10 —3	14	12	13	14	14	14	14
10 —4	16	14	16	16	16	15	16
10 —5	12	12	12	12	12	12	12
10 —6	..	..	..	..	..	..	..
	2	1	2	2	2	2	2

* Doubtful.

N. B.—All the samples giving positive presumptive results including the two doubtful cases were positive to the confirmatory (completed) test.

A fairly good agreement seems to exist between the four solid media as far as the results of the presumptive test are concerned. Very clear-cut results have been obtained on MacConkey's agar and violet red bile agar, both of which showed well developed and typical colonies of coliform organisms with very few gram-positive and other extraneous organisms growing on them. The intruding types were generally found to be staphylococci, micrococci and corynebacteria. Although typical colonies of coliforms were formed on the other two media (Endo's and eosin methyl blue agars), in many instances the medium was found blown up due to gas production making it difficult to obtain an accurate count on them. In the case of eosin methylene blue agar particularly, too many extraneous organisms (belonging to the types mentioned above) were found to be growing on the plates, causing considerable difficulty in recognition and enumeration and also possibly interfering with the development of coliform colonies.

TABLE II

Comparative efficiency of different media for grading milks by the presumptive coliform test

Type of milk	Coliform bacilli Absent (ml.)	Number of samples indicated in different media							
		present (ml.)	Mc. Broth	F.R. Broth	B.G.S. Broth	Mc. Agar	Endo's Agar	E.M.B. Agar	V.R.B. Agar
Pasteurised milk . . .	10^{0}	10^{0}	2	3	2	5	4	5	5
	10^{-1}	10^{0}	4	3	4	1	2	1	1
Farm produced milk . .	10^{-2}	10^{-1}	4	5	6	7	7	7	7
	10^{-3}	10^{-2}	12	11	10	9	9	9	9
	10^{-2}	10^{-1}	2	..	2	2	4	2	3
Village milk	10^{-3}	10^{-2}	2	4	4	8	3	4	4
	10^{-4}	10^{-3}	9	9	7	3	6	7	6
Bulk milk (Private dairies) .	10^{-3}	10^{-2}	2	2	2	2	3	2	4
	10^{-4}	10^{-3}	4	4	5	6	5	6	5
	10^{-5}	10^{-4}	4	4	3	2	2	2	2
	..	10^{-5}	8	8	8	8	8	8	7

Since the minimal quantity of milk showing the presence of coliform organisms is taken as the criterion for assessing the extent of contamination for grading purposes, the comparative efficiency of each medium from this point of view has been examined in Table II. The samples have been grouped according to their source and general level of bacteriological quality, and the relative proportions of positives in the highest dilutions of each series indicated by the different media are shown. Even when judged from this angle, the liquid media compare well with each other and about 85 per cent of all the samples tested are placed in the same grades by each of the media as indicated by the results of the presumptive test in the four groups. All the three broths may, therefore, be said to be equally efficient for the presumptive coliform test in milk, but as MacConkey's broths is more familiar and can be easily prepared in any laboratory from ingredients available locally, it offers some advantages over the other two media for use in this country.

Similar agreement is found among the solid media, although in one case (village milk) MacConkey's agar has given a smaller number of positives than the others in the highest dilution of the series, which may be due to sampling errors or to a higher inhibitory character of the former medium. Though the four media have yielded fairly comparable results from the point of view of the presumptive coliform test, MacConkey's and violet red bile agars have been found more satisfactory for the purpose than either Endo's or Rosin methylene blue agar due to the drawbacks pointed out earlier in respect of the latter two media. The latter have, however, proved useful for confirmatory studies. In the opinion of the authors, MacConkey' s agar is most suitable as it has been found to give highly typical and clear colonies of coliform organisms and permit very few non-coliforms to develop on the plates.

From a comparison of the solid with liquid media, it is observed that the results do not agree so closely and only about 72 per cent of the samples are placed in the same grades by all the media. The broths have generally indicated a greater number of positives than the solid media in the highest dilutions of each series and a good number of samples (20 per cent) found positive in the former are recorded as negative and thus up-graded by the latter media. It cannot be definitely concluded from this that the solid media give an erroneous estimate of the coliform content, although they may be considered to exert a slightly greater inhibitory effect on the coliform organisms and present a more conservative picture than the liquid media. It is well known that the dilution method of counting bacteria is subject to very large sampling errors even when several tubes are used in each dilution and that the results must be regarded as only approximate [Halvorson and Ziegler, 1933; Wilson *et al.*, loc. cit.]. Accordingly a positive presumptive evidence based on the appearance of five or more typical colonies of coliform organisms on a single or duplicate set of plates may be considered to be as reliable as that obtained from the results of fermentation observed in a number of broth tubes. The plating method is much simpler and gives clear results in 24 hours, whereas the

broth tubes require incubation for 48 hours according to the recommended official procedure [Ministry of Agriculture, 1934], although in the present experiment even the liquid media have yielded definite results in 24 hours.

SUMMARY

The use of different liquid media (MacConkey's, formate ricinoleate and brilliant green bile broths) and solid media (MacConkey's, Endo's, Rosin methylene blue and violet red bile agars) for the estimation of coliform organisms in Indian milks has been compared.

2. The results of the presumptive coliform test in liquid media (dilution method) and solid media (plating method) confirmed on Endo's or Rosin methylene blue agars have been examined and the comparative efficiency of each medium brought out. All the three liquid media and MacConkey's and violet red bile agars among solid media have been found to give satisfactory results in 24 hours.

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THE VARIATIONS OF ACIDITY, ALCOHOL TEST AND PHOSPHATE NUMBER IN SAMPLES OF FRESH BUFFALO MILK

By Panwar Gaj Raj Singh, T. M. Paul and C. P. Anantakrishnan, Indian Dairy Research Institute, Bangalore

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Generally milk can withstand high temperature without undergoing any unwelcome change and this is a very important factor both from the manufacturers' as well as the consumers' points of view. But, it is not very uncommon that sometimes certain samples of milk do not stand even the ordinary boiling. To determine the hygienic quality as well as to assess the suitability or otherwise of the different lots of milk arriving on a factory platform for various methods of processing different platform tests have been suggested from time to time. The boiling test, acidity determination, alcohol test and phosphate test are the chief among them. The boiling test when positive, indicates that the milk has developed rather high acidity, which removes calcium from combination with the protein, and therefore allows casein to settle. Boiling only accelerates the last phase of this reaction. Acidity determination, as it is usually done, by titration against decinormal sodium hydroxide, does not give a correct picture of the true acidity of a complex fluid like milk according to Rogers *et al.* [1921]. Even the true acidity of milk as determined by *p*H value is not a criterion in ascertaining the heat stability of milk according to the same authors. Again, acidity of milk from individual animals, is subject to considerable variation and the value of acidity determination becomes very questionable. Thus the usefulness of the acidity test is limited to the detection of milk that has developed considerable amount of acidity as a result of bacteriological action caused by unhygienic ways of production. The alcohol test has been commonly used for weeding out milk produced under unhygienic conditions, as well as those that are unstable to heat. According to Sommer and Binny [1923] this test is subject to the influence of various factors, so much so, that its usefulness is very restricted. The findings of Dahlberg and Garner [1921] do not guarantee that this could always be used as a criterion of the heat stability of milk. The effects of acidity and the concentration of salts are additive so far as the alcohol test is concerned, but Rogers and Deyshire [1921] and Sommer and Hart [1926] suggest that the concentration of the mineral salts forms only a minor factor in determining the heat stability of a milk. In fact no test which could predict the heat stability of condensed milk with a reasonable amount of certainty was known till Ramsdell *et al.* [1931] developed the phosphate test. The above authors have reported a phosphate number ranging from 12 to 37 for single herd milk samples from Freisian and Channel Island breeds and composite milk samples. According to their findings, milk samples with a phosphate number below 20 coagulated within four minutes during sterilization, while samples with a phosphate number above 20 had greater heat stability, but at the same time, they warn that a high pho phate number does not always indicate a highly heat resistant milk sample.

Although there is no local or even seasonal surplus of milk in India for the manufacture of condensed milk, during the last war various isolated attempts were made in certain places to manufacture condensed milk out of buffalo milk. Anantakrishnan and Kothavalla (*unpublished work*) have reported that the alcohol test is more reliable than the phosphate test in predicting the suitability of buffalo milk for condensing and sterilization, but this is just the contrary of the findings of Rams dell *et al.* [1931]. Probably this difference might be due to certain peculiarities in the phosphate number of buffalo milk as distinct from cow's milk which alone was investigated by the foreign authors. Therefore a study of the seasonal and lactational variations in the phosphate number with special reference to its relation to other platform tests like acidity and alcohol tests was undertaken and the results of the investigation are presented in the following pages.

Experimental

Six Murrah buffaloes, all in the first month of lactation, were selected from the herd of the Indian Dairy Research Institute. Milk samples were collected in the morning and tested for acidity, alcohol

test and phosphate test at 9 a.m. Acidity was determined by the usual titration against standard solution of sodium hydroxide with phenolpthalein as indicator. The alcohol test was carried out as usual by mixing equal amounts of alcohol and milk (2 ml.) but the strength of the alcohol was varied from 60 to 80 per cent by volume till a positive test was obtained. The method of Ramsdell *et al.* [1931] was adopted for the phosphate number, viz., by adding different amounts of 0·5 M. solution (68·1 gm. per litre) of potassium dihydrogen phosphate to 2 c.c. lots of milk till coagulation occurred within 0·02 ml. of the reagent on heating the mixture in a boiling water bath for five minutes. Any coagulation was taken as positive and the phosphate number was calculated by multiplying the amount of the reagent expressed in fractional millilitres by 100. The tests were carried out for about one complete lactation to study the seasonal and lactational variations in the various tests if there were any. Also two composite samples, one cow and one buffalo, were tested daily for acidity, alcohol and phosphate tests. Altogether about 1,500 tests were made, each for phosphate number, acidity and alcohol tests. In the case of the phosphate number, it was a very tedious process to arrive at the actual amount of phosphate solution required to produce coagulation as the phosphate number varied between 8 and 80. Almost a similar difficulty was experienced in the case of the alcohol test, where also there was considerable variation from sample to sample. The weekly average of the phosphate number for the individual buffalo milk samples as well as the two composite milk samples are presented in Table I. The relationship between acidity and phosphate number, acidity and alcohol test and alcohol test and pho phate number are indicated in Tables II, III and IV respectively.

TABLE I

Weekly average of the Phosphate numbers of buffalo milk

Week	Buffalo 1	Buffalo 2	Buffalo 3	Buffalo 4	Buffalo 5	Buffalo 6	Average of six	Buffalo composite	Cow composite
1st . .	28	26	31	20	44	26	29	32	28
2nd . .	27	26	39	25	51	26	32	30	47
3rd . .	27	25	45	24	50	30	33	33	30
4th . .	28	25	45	25	62	28	35	35	32
5th . .	29	31	50	22	66	29	38	36	31
6th . .	28	24	60	20	58	24	36	38	30
7th . .	26	23	48	21	57	25	33	37	32
8th . .	25	25	55	21	63	27	36	40	33
9th . .	21	28	54	22	66	32	38	39	38
10th . .	26	24	51	21	66	29	36	41	34
11th . .	30	36	68	32	64	36	44	40	35
12th . .	26	33	71	23	72	37	44	38	34
13th . .	28	39	68	24	61	35	42	36	35
14th . .	26	30	65	26	65	36	41	41	30
15th . .	30	28	64	27	65	33	41	34	32
16th . .	29	30	59	24	59	31	39	37	29
17th . .	30	33	54	26	57	35	39	33	28
18th . .	30	34	60	25	62	37	41	35	28
19th . .	35	34	58	25	58	32	40	36	29
20th . .	32	31	54	26	58	28	38	35	28
21st . .	34	24	63	29	53	33	39	33	30
Average .	29	29	55	24	60	31	38	36	31

DISCUSSION

Acidity

There are considerable variations observed in the acidity of fresh individual samples of buffalo milk and even for the same individual sample; day to day variations are so considerable that they cannot be attributed to experimental error. The average acidity of fresh buffalo milk in the first

month of lactation is found to be 0·12 per cent expressed as lactic acid but there is a slight gradual increase in titratable acidity as the lactation advances; this is more pronounced in certain cases while it is not so evident in other cases. Probably this increase in titratable acidity is not due to true acidity but due to other factors like casein and phosphates which also combine with alkali as suggested by Rogers *et al.* [1921].

TABLE II

Acidity of milk and phosphate test

Phosphate Numbers	Acidity in milk (percentage of lactic acid)										
	0·10	0·11	0·12	0·13	0·14	0·15	0·16	0·17	0·18	0·19	Total
0—15	..	..	5	33	7	18	5	14	9	9	100
15—25	5	17	26	42	49	38	44	25	16	6	268
25—35	13	50	68	55	73	49	21	6	11	33	379
35—45	1	11	21	21	18	9	13	5	9	4	112
45—55	5	6	15	4	11	10	13	4	2	2	72
55—65	6	20	29	13	14	14	12	4	7	1	120
65—75	10	17	8	4	8	8	4	..	..	1	60
75—85	..	..	1	..	..	..	..	..	..	..	1
Total	40	121	173	172	180	146	112	58	54	56	1112

Alcohol test

The test has been ordinarily used for assessing the hygienic quality of milk, although the reliability of the same has been questioned by Sommer and Binny [1923]. The concentration of the alcohol used is also not very definite, various concentrations ranging from 68 to 75 per cent by volume being used by different workers. From Table III it is quite clear that positive alcohol test for the same concentration of alcohol is distributed over a wide range of acidity of milk. This naturally indicates that factors other than acidity come into play in making an alcohol test positive. But the general tendency, as is evident from Table III, is that as acidity increases, greater percentages of samples give a positive alcohol test with the same concentration of alcohol and this is found to be statistically significant. Explanations for any disagreement with this general finding, have to be sought in other factors like concentration of mineral salts, rennet forming organisms, etc., as suggested by Sommer and Binny [1923]. Another point of interest evident from Table III is the unusually high number of fresh milk samples that gave a positive alcohol test with 60 per cent alcohol. Composite cow milk that was examined side by side very seldom gave a positive alcohol test below 70 per cent while composite buffalo milk in every case gave positive alcohol test almost always below 65 per cent. Therefore, it is clear that the present concentrations of alcohol generally used in the case of cow's milk are too strong for buffalo milk. Taking the average acidity of fresh buffalo milk as 0·13 per cent and considering Table III, it is seen that about 42 per cent of the samples gave positive alcohol test with 60 per cent alcohol. Perhaps this peculiar behaviour has to be attributed to some difference in salt balance in the case of buffalo milk as distinct from cow's milk. As the acidity increases most of the samples give positive alcohol test with 60 per cent alcohol. Acidities like 0·16 and 0·17 per cent are sometimes observed in fresh buffalo milk and in such cases 70 and 85 per cent respectively give positive alcohol test with 60 per cent alcohol. Whatever be the reasons for this, it is quite clear that in the case of the alcohol test for buffalo milk, the concentration of alcohol has to be reduced to 60 per cent. Even at 0·13 per cent acidity there are samples which do not give a positive alcohol test even with 80 per cent alcohol, but the number of such sample is very few. This also may be attributed to some change in salt balance.

Phosphate test

Percentage of Alcohol	Acidity of milk (percentage of lactic acid)										
	0·10	0·11	0·12	0·13	0·14	0·15	0·16	0·17	0·18	0·19	Total
60	3	18	42	66	86	95	87	58	54	39	548
62	2	9	16	25	29	20	11	1	4	..	117
65	6	20	22	23	22	15	12	1	3	2	126
68	3	10	18	11	19	5	3	1	2	..	72
70	5	14	18	13	8	5	7	2	1	..	73
72	3	11	12	7	9	5	3	..	..	..	50
75	5	11	22	7	6	4	5	..	..	..	60
78	6	4	4	5	5	2	..	..	..	..	26
80	1	7	5	..	..	..	..	..	..	..	13
Total	34	104	159	157	184	151	128	63	64	41	1,085

A glance at Table II will clearly show the wide variations in the distribution of the phosphate numbers. Although no consistent relationship between titratable acidity and phosphate number has been reported by the foreign workers, it is quite clear that the phosphate number comes down as the acidity increases. At an acidity of 0·10 per cent lactic acid in milk, only below one per cent of the readings for phosphate number fall below 20. As the acidity increases the percentage of phosphate numbers below 20 increases rather rapidly so that at an acidity of 0·13 per cent, 27 per cent of the readings fall below 20, and 56 per cent at an acidity of 0·19 per cent. Therefore, it is quite clear that there is some relationship between acidity and phosphate number, and this too is found to be statistically significant.

TABLE III

Acidity of milk and alcohol test

TABLE IV

Alcohol test and phosphate numbers

Phosphate Number	Alcohol concentration									
	60	62	65	68	70	72	75	78	80	Total
0—15	32	..	..	..	..	..	..	..	..	32
15—25	66	18	12	9	6	1	1	2	..	115
25—35	56	60	71	36	30	12	18	9	2	294
35—45	13	27	31	6	12	9	9	4	..	111
45—55	3	5	13	2	3	2	7	2	..	37
55—65	7	12	8	4	9	6	17	9	5	77
65—75	1	7	4	..	2	7	8	2	2	33
75—85	..	1	..	..	..	..	..	..	..	1
Total	178	130	139	57	62	37	60	28	9	700

Table IV represents the relative distribution of alcohol and phosphate tests in the same field. In the case of samples that give a positive alcohol test with 60 per cent alcohol, the proportion of samples that have a phosphate number of 20 or below is as high as 39 per cent. With samples that give positive alcohol test with 62 per cent alcohol, the percentage of samples giving a phosphate

number below 20 is as low as 5, and beyond 62 per cent alcohol this percentage is negligible. In the alcohol test it is a common observation that as the quality of milk deteriorates the sample gives a p itive alcohol test with lower and lower concentration of alcohol. Similarly Ramsdell *et al.* [1931] have reported that samples with phosphate number below 20 are less stable to heat. In view of the findings of the present investigation these two tests could be put together to assess the quality of the milk sample under investigation.

Table I shows the individual and other variations in the phosphate number so far as buffalo milk is concerned. For fresh individual samples of milk, figures as low as 10 and as high as 82 have been met with, but such figures are very few, so that the mean is always far from both of these. In most of the individual samples, it is found, that there is a gradual increase in the phosphate number as the lactation advances, although this increase is not maintained towards the end of the lactation. Almost the same tendency is observed in the case of the composite cow and buffalo samples. There is considerable daily variation in the phosphate number of the individual milk samples although this is not so very marked in the case of the composite sample. The weekly average of the phosphate numbers has never been below 20, although it has touched 20 at times. This shows that there are at least a few samples which fall below 20. Ramsdell *et al.* [1931] have reported that cow milk samples with phosphate numbers below 20 have a low heat stability. From Table I it is seen that buffalo composite milk has got a higher phosphate number when compared to cow composite milk. Probably this may be due to the difference in salt distribution balance.

SUMMARY

1. The various commonly used platform tests like the acidity, alcohol and phosphate tests have been studied from the beginning of one lactation for nearly six months in the case of individual buffalo milk samples.

2. The inter-relationship between the various tests has been investigated and discussed.

3. There is considerable variation in the acidity of the samples from individual to individual and from day to day.

4. The alcohol test requires modification by way of reducing the concentration of the alcohol, to be used for buffalo milk. A concentration of 60 per cent alcohol by volume is recommended for buffalo milk.

5. There is found to be wide variation in the phosphate number of individual buffalo milk samples.

6. An increasing number of samples with high titratable acidity have a phosphate number below 20.

7. A good proportion of the samples giving a positive alcohol test with 60 per cent alcohol were found to have a phosphate number 20 or below.

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AN OUTBREAK OF GOAT-POX IN HISSAR DISTRICT (PUNJAB)

By H. K. Lall, B. Sc., M. R. C. V. S., Gopal Singh L.V.P, and Jagat Singh, L.V.P.,
Government Livestock Farm, Hissar

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OUTBREAKS of goat-pox have been reported from time to time from various provinces and States of India, but as compared to sheep-pox its occurrence has been less conspicuous. Three cases of goat-pox were recorded in 1929-30 at the Government Livestock Farm, Hissar, but there isno reliable evidence of its recurrence until 1944-45 when the disease spread to farm goats from the experimental goats.

The present work arose out of an outbreak of this disease in Hissar district. In January, 1945, the disease was reported from Dabwali but no material was available for investigation. A month later, report of a fresh outbreak was received from Ratia, Sarsa and Fatehabad villages to the east of Dabwali. The disease has been observed in the other parts of Punjab in winter or early spring; sheep-pox has a similar seasonal trend. Such a trend has not been mentioned in the various Disease Investigation Officers' reports. Chadha [1942-43] has, however, stated in connection with an outbreak in Bombay in 1942-43 that the disease prevailed throughout the year.

The goats affected in the outbreak in Ratia were of the ordinary *desi* type. The various flock owners reported the mortality as 19 per cent in goats and 11·4 per cent in sheep, but whether all this illness was due to goat-pox cannot be definitely said. Most of the animals examined by us were recovering and showed pox nodules in the form of haemorrhagic scabs over the back and flanks. Two examined *post-mortem* showed no internal lesions except for a single lung nodule in one of them. One female kid had pox lesions all over the body including the udder, the size of discrete nodules varying from that of a 4 anna to 8 anna bit. To study the disease in detail, a kid having pox nodules was brought to Hissar.

Experiments at Hissar

Transmission work

Preliminary experiments were carried out in one of the farm blocks, about 1½ miles from the main dairy flock. The disease was transmitted to two six-month old kids (Nos. 1 and 2), using a suspension of scab material from the diseased kid. This suspension was scarified on the abdomen and a drop of it was also injected intracutaneously. Two other kids, three months old (Nos. 3 and 4), one of which was of hill breed, were then infected from material taken from kids 1 and 2. To maintain the virus for further study, yet another kid was infected with virus from Nos. 3 and 4, but it failed to react and we presumed that the virus was lost. On previous occasions also some difficulty has been experienced in establishing the disease under experimental conditions. A similar experience has been expressed by Henderson [1938].

None of the inoculated kids developed the disease in generalized form. Iyer [1939] has reported successful experimental reproduction of goat pox in a generalized form by inoculating sub-cutaneously blood taken at the height of fever or by means of spleen suspension. However, these methods were not tried by us. Following the cutaneous inoculations in our experimental kids, only a local reaction was observed. Four or five days after the inoculation, there was a slight reddening of the scarified area, corresponding with a rise of body temperature. After eleven days, Kid No. 1 showed sharply defined vesicles. The vesicles were flattened and firm to the touch, and had little fluid in them. In kid No. 2 there was a rather firm prominent swelling over the scarified area, up to the size of the palm of the hand. Within the next few days the lesions in these two animals became very hard and nodular and were covered with haemorrahagic scabs. The vesicular stage was very transient. The temperature rose by nearly 2°—3°F. on the fifth day and maintained that level for 11 or 12 days.

Spread of the disease

Some adult goats, which were being kept for another experiment in the same enclosure as the pox-infected kids, were transferred to the main goat dairy one month after the last transmission experiment. These goats did not catch the infection during five to six weeks they were in contact with the artificially infected kids, and it was therefore presumed they were healthy. However, six weeks after the adult goats were transferred, cases of goat-pox were detected among them and also among the dairy goats. After the disease had subsided in the female stock, it spread to th males which were being kept away from the dairy goats, to avoid infection. These observations seem to suggest that the incubation period in goat-pox may be fairly long. According to Melanidi and Tzortzaki [1936], the incubation period in goat-pox is two to three days, followed by a stage of skin eruption lasting for two to three weeks.

Mortality and lesions

From the reports of Disease Investigation Officers [1936-1945], it is found that in Bombay, in 1938-39, there was 9 per cent mortality in one outbreak and 73 per cent in another ; two outbreaks recorded in the United Provinces in 1938-39 and 1942-43 showed a mortality-rate varying from 15 to 20 per cent. Vishwanathan [1938-39] reported a 30 per cent mortality-rate in an outbreak diagnosed in 1938-39. Kuppaswamy [1936] reports a mortality rate of 54 per cent attributing the high percentage to unfavourable climatic conditions, lack of hygiene and unsuitable food. According to Donation and Lestoquard [1936] discrepancies in the results of different workers on sheep-pox are due to differences in the susceptibility of individual sheep and state of the virus, which has three distinct phases of virulence from extreme to low. This is probably true in the case of goat-pox as well. Altogether, in the original outbreak, 11 out of 179 female goats (39·7 per cent), 3 out of 13 male goats, 25 out of 100 kids (25 per cent), and 15 out of 30 (50 per cent) castrated kids, males, suffered from the disease. The incidence was less in females and kids than in males and adults. The outbreak lasted for about two months. There were only two deaths, viz. goat No. 59, which developed gangreaous pneumonia as a complication, and an Angora buck which showed pox lesions in the lung. Mortality was, therefore, low in this outbreak as compared with that reported by the Disease Investigation Officers [1936-45] possibly due to low virulence of the virus.

In most cases pox nodules were generalized over the body, the teats and scrotum being particularly affected. There was rise of temperature lasting for 10 days or more, and considerable decline in the milk yield during the active phase of the outbreak. A typical acute case was that of goat No. 929. Vesicles were present all over the body, nostils, lips, udder and teats. Pneumonia developed with rise of temperature to 107°F. This goat, however, recovered after a laborious treatment. In some cases there was suspension of rumination, and rapid respiration. A tendency to sit in the sun was observed in many cases. On the seventh day vesicles became black and by the ninth day scabs had formed.

SUSCEPTIBILITY OF OTHER ANIMALS

According to the flock owners, sheep also died during the outbreak, but no affected sheep were seen at the time of the visit. From the reports of the various Disease Investigation Officers [1936-45] it seems that in outbreaks of goat-pox only goats were affected, though it is not clearly stated in all cases that these were the only animals affected. According to Melanidi *et al.* [1936], cattle, horses, rabbits and poultry are also susceptible to the virus of goat-pox while man is insusceptible, but it is only in goats that the disease takes an epizootic form. Slagsvold [1938], on the other hand, states that disease is readily transmissible among goats and sheep, and attempts to infect cattle, pigs, horses, rabbits, guinea-pigs and poultry failed. Bennett, Horgan and Haseeb [1944] obtained a typical pox syndrome in sheep, cows, and rabbits with goat-pox virus, though they found no immunological relationship between the viruses of vaccinia, sheep-pox and goat-pox. A close relationship, however, was observed between the viruses of goat-pox and contagious pustular dermatitis of goats. Hutyra and Marek [1926] have also stated that in some cases transmission of goat-pox to sheep is successful but that only an incomplete pox exanthema is produced at the point of inoculation.

In the first instance we tried without success to infect two lambs, a buffalo calf and a cow calf. Some Hissari and Bikaneri sheep were also constantly kept in close contact with infected animals but the disease did not appear among them. In another attempt two Bikaneri, two Hissari (Merino-Bikaneri crosses) lambs were infected; in one animal of each bread the virus was scarified and in the other it was given intracutaneously. The two scarified lambs developed vesicles over the scarified area but nothing happened in the other two. The experiment was repeated with two more lambs using udder scabs from a severe case, goat No. 59. One of the two lambs, No. 248 was scarified as well as inoculated intracutaneously at the caudal fold, while in the other, No. 272, the material was given intracutaneously only. No. 248 developed a local reaction and pox lesions on the sixth day on the scarified area only, while the other developed vesicles on the abdomen at about the same time. These experiments indicate that it is possible to produce a localized lesion in sheep with goat-pox virus, but that sheep do not get a generalized form of the disease either by artificial infection or by exposure to naturally diseased animals.

A group of cross-bred hill goats, maintained at the farm (Kangra hill Angora) contracted the disease naturally and, of course, it was also possible to transmit the disease to them artificially. Out of the two cross-bred kids used for experiment, one was scarified on the abdomen and the other was injected intracutaneously at the caudal fold. The kid which was scarified developed local vesicles on the third day and a generalized form of the disease on the eighth day, while the other failed to develop the disease except for a local reaction in the form of a hard nodule.

VACCINATION

When the first case was brought to our notice, it was deemed necessary to immunize the rest of the flock, for which purpose serum-sensitized and glycerinated virus vaccines were tried. The virus was obtained from field cases. This method along with 'ovination' has been tried by Chadha [1942-1943] with inconclusive results. Immunization by ovination was also tried on 510 goats by Vishwanathan [1938-1939] who observed severe local or generalized reaction in most of them.

Glycerinated virus

Fifteen female goats 12 to 15 months old and ten kids two to three months old were inoculated intracutaneously at the caudal fold on the 19th April, 1945, with two drops of glycerinated virus (virus material mixed with an equal volume of glycerine) which had been left for 24 hours at serum pit temperature (about 55-65°F.). The vaccinated animals, with one exception, developed a typical local reaction in the form of a flat, sharply circumscribed vesicle of the size of an eight anna bit. The reaction started as a reddening on the third day, developing into a raised flat vesicle two or three days later. In three cases, where an excess of virus was injected by accident, the local reaction was severe, resulting in necrosis and ulceration of the skin. Five female goats also developed a few lesions on the body, but these animals were possibly in the incubation stage at the time of vaccination. Mild thermal reaction lasting for three to four days was seen in some cases on the sixth day.

Serum-sensitized virus

Scabs from fresh cases were soaked in serum from a recovered animal, who had shown an acute infection and the suspension kept in the serum pit for 24 to 48 hours. Ten kids were injected intracutaneously into the caudal fold with suspension which had been held for 24 hours, and this produced a local reaction in all except one. Three of the kids developed a mild degree of pox with two to three vesicles only. In a second group of five goats, which were given a 48-hour old suspension, there was a local reaction only. These reactions were similar to, though slightly milder than the ones produced by glycerinated virus, and consisted of a rise of temperature on the fourth to the sixth day.

A week after the reaction had subsided, all the vaccinated animals along with five controls, were kept in close contact with infected goats for three weeks, but unfortunately neither controls nor vaccinated showed any symptoms. These experiments were thus inconclusive, except for the fact that neither glycerinated nor serum-sensitized virus produced severe reactions when used as a vaccine.

Chadda [1942-1943] however, obtained unexpectedly severe reactions in one of his experiments and believed this to be due to the serum not being sufficiently protective.

Immunity test

Ten female goats, obtained from a goat-pox-free area, were successfully vaccinated with glycerinated virus on the 22nd June 1945. These were kept for the purpose of immunity testing. Unfortunately, at this time, no goats could be vaccinated with serum-sensitized virus due to lack of facilities. Nearly six months later, eight of these goats, together with four kids four months old and born after the outbreak subsided, acting a controls, were scarified on the abdomen with a saline suspension of goat-pox virus obtained from the Indian Veterinary Research Institute, Mukteswar. Marked local and thermal reaction (rise of 2°F.) was noticed on the fifth day in the vaccinated as well as in control animals. On the tenth day the temperature returned to normal, and the local reactions subsided after 20 days. The results were thus again inconclusive. Apparently, the Mukteswar virus was not very virulent, as none of the animals was severely affected. The present experiments, as well as those of other workers in India, indicate the need for further work on the virulence and potency of goat-pox virus in India, its relation to that of sheep-pox and the methods of combating the disease in the field.

Summary

An outbreak of goat-pox is described in the dairy flock of goats at the Government Livestock Farm. The disease spread from animals experimentally infected with virus obtained from an outbreak in Hissar district. Mortality was low, but there was considerable reduction in milk yield as the result of the disease. Attempts to produce the disease in a calf and a buffalo calf were unsuccessful. It was, however, possible to produce local lesions in lambs with the goat-pox virus. Groups of goats vaccinated with serum-sensitized or with glycerinated virus, along with non-vaccinated controls were exposed to natural infection immediately after vaccination, but neither the controls nor the vaccinated ones developed the disease. An immunity test, carried out on another group of goats six months after vaccination, with glycerinated virus, was also inconclusive, because both vaccinated and control goats reacted with goat-pox virus obtained from the Indian Veterinary Research Institute, Mukteswar.

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SOME OBSERVATIONS OF PASTEURELLOSIS

I.—AN OUTBREAK OF PASTEURELLOSIS IN GEESE

By R. N. Mohan, Veterinary Investigation Officer and S. K. Bhadury, B.Sc., Assistant Veterinary Investigation Officer (Poultry), Bengal

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RECORDS of the incidence of pasteurellosis in birds in India are limited to fowls and the occurrence of natural outbreaks of the disease in geese and other poultry have received little attention. In this paper is recorded, presumably for the first time in this country, an outbreak of 'fowl cholera' in geese. The diagnosis was made by microscopical and cultural examination. Some pathogenicity tests were also done.

History of the outbreak

Geese were purchased periodically from different places in the mofussil and stocked near Calcutta for sale in the city and elsewhere. At the time of investigation, 6th December, 1945, there were 1325 geese and a few ducks. These were accommodated under most unhygienic conditions on an area measuring about one-quarter of an acre, which included a small pond, so that there was considerable over-crowding.

Sporadic deaths were more or less a regular feature, but after the middle of November, 1945 the geese started dying in increasing numbers. Thus, during the 12 days preceding investigation, some 160 geese died, 24 deaths being reported on the last day.

Only one duck died late during the period of investigation. This too was diagnosed as pasteurellosis on blood-film examination.

Investigation

Microscopical examination of blood-films and post-mortem examination of one goose cadaver received with the mortality report on the 6th December, led to the diagnosis of pasteurellosis. This was confirmed on further investigation, the results of which are given below.

Microscopical examination. Films from peripheral and heart blood, and also from liver and spleen, of several cadavers constantly revealed 'bipolar' organisms, typical of *Pasteurella aviseptica*, which were generally present in large numbers. In some cases, even in Leishman-stained films, there were distinct indications of the presence of capsules manifested by a clear halo around the organisms, in contrast with other post-mortem invaders which sometimes gained access in small numbers.

Cultural examination. From heart blood and liver of two cadavers *Pasteurella aviseptica* was isolated in pure or almost pure cultures. Morphologically, they were Gram-negative short rods and ovals. Their other cultural and biochemical characters were: non-motile; small, low convex, round, transluscent, butyrous colonies on agar plates; fairly good growth with incomplete surface pellicle, turbidity and viscous sediment in broth; moderate growth on agar slope; good growth on blood-agar; no haemolysis; no growth on McConkey; no change in litmus-milk; indole strongly positive; acid, no gas, in sucrose, mannitol and maltose, but not in lactose.

Pathogenicity tests. Rabbits, guinea-pigs and fowls were available for inoculation. These were used as follows:

About 2 c.c. of heart blood from a goose cadaver was mixed with about 5 c.c. of sterile
Of this about 3 c.c. was inoculated subcutaneously into a rabbit and about 2 c.c. intraperit
into a guinea-pig. Both died the following night and films from peripheral blood and
organs showed typical *Pasteurella*.

A few drops of heart blood from the rabbit were diluted with about 5 c.c. of sterile broth and inoculated subcutaneously into another rabbit and intravenously into a fowl. Again, both died the following night and *pasteurella* was demonstrated in large numbers in films and in cultures from their heart blood.

Similarly, from the guinea-pig, heart blood was subinoculated intravenously into a fowl and subcutaneously into another guinea-pig. The second guinea-pig looked sickly for some days, but survived. The fowl died about 40 hours after inoculation and showed typical *pasteurella* in peripheral blood, heart blood, liver and spleen films.

Clinical features. These were not observed systematically. The symptoms noted were: marked dullness, loss of appetite, increased thirst, some discharge from the beak, diarrhoea, and in a few cases weak limbs probably due to involvement of joints.

Post-mortem findings. According to the post-mortem picture most cases would fall in the class of sub-acute infection. Thus, besides usual haemorrhages and varying degrees of enteritis, the liver was found studded with numerous minute discrete grayish-yellow 'necrotic' foci. A few livers examined earlier in the outbreak appeared clean, except being slightly enlarged, while in a few case, examined later in the outbreak, larger 'necrotic' areas were noted. The spleen was never found distinctly enlarged.

Control measures

The obvious preliminary step was to relieve overcrowding. This could not be accomplished for want of space. Such other sanitary measures, like disinfection of premises, etc. were adopted as the prevailing conditions permitted.

Inoculation with fowl cholera vaccine and serum as prepared by the Indian Veterinary Research Institute was undertaken. Since the owners could not be persuaded to withhold sale beyond a few days, the results of these measures could not be correctly assessed. Deaths continued to occur during the first week after inoculation, but later the disease seemed to have been brought under control.

Acknowledgements

The cultural and biochemical tests were done in the School of Tropical Medicine, Calcutta. The facilities granted by the Director, Lt.-Col. Pasricha, are gratefully acknowledged. The authors are also grateful to Mr. J. R. Haddow, Veterinary Adviser to the Government of Bengal, for his interest in the work.

II.—PASTEURELLOSIS IN DUCKLINGS

By S. Nurul Mohteda, G.M.V.C., Assistant Disease Investigation Officer (Poultry), Hyderabad Dn.

(Received for publication on 14 October 1946)

FOWL CHOLERA, as it affects ducklings three to four months old, was studied for the first time in Hyderabad State in 1943. A general survey of poultry disease in the State revealed the existence of sickness affecting ducks which caused sudden death and which was suspected to be either cholera or paratyphoid infection. The disease usually subsided of its own accord. In the present investigation the death of 12 ducks was reported within four days of their purchase. No relevant information concerning the source of the disease was available. One ailing and one dead bird were brought to the laboratory for examination.

Lesions. No diagnostic symptom of pasteurella infection was apparent. Pinpoint haemorrhages were found in the heart coronary fat; the lungs were congested and partly pneumonic; the liver contained petechial haemorrhages, as well as white necrotic foci, while the intestines were affected with catarrhal haemorrhagic enteritis. All other organs were normal.

Diagnosis. Films of heart-blood and liver contained bipolar organisms simulating *Pasteurella aviseptica*. To confirm the diagnosis a pigeon was infected intraperitoneally with 2 c.c. of the suspension of the liver, spleen and heart-blood, while a fowl was infected intravenously with the same amount of suspension of bone marrow. Both died after 18-20 hours and smears of this blood contained organisms indistinguishable from those of *P. aviseptica*. Again, another fowl was infected intravenously with the same results. A rabbit inoculated intraperitoneally with 1 c.c. of heart-blood died within five hours of the injection after struggling for 5-10 minutes and passing semi-solid faeces. In this case also blood smears were positive for *P. aviseptica*. The material from the original case as well as from the experimental birds were sent to Izatnagar for bacteriological study. The material included demuscled femur and tibia bones preserved in powdered charcoal, a portion of the liver, a heart-blood swab, and heart-blood in a pipette. Pasteurella infection was confirmed. The organism isolated was pathogenic to fowl, duck, pigeon and rabbit, but cross agglutination tests with various types of sera proved it to be different from the usual fowl strain.

Summary

An outbreak of fowl cholera or pasteurellosis among ducklings in an endemic form has been recorded in Hyderabad State. The duck strain (*Past. aviseptica*) on serological examination revealed significant differences in its antigenic composition from that of the chicken strain.

III.—AN OUTBREAK OF PASTEURELLOSIS IN DUCKS

By S. K. Bhadury, B.Sc., Assistant Disease Investigation Officer (Poultry), Bengal

(Received for publication on 20 January 1947)

THE disease, though not uncommon, seems to have been little described so far as concerns symptoms, post-mortem features and other correlated aspects of diagnosis and control. The present article deals with a subacute type of outbreak in Bengal. Pasteurellosis was first suspected from microscopical examination of films prepared from the heart blood of affected birds, and diagnosis was subsequently confirmed by biological test. A significant point was that the causal organisms could be demonstrated only in birds in which the course of the disease was prolonged. The characteristic liver lesions, viz. greyish necrotic foci, were a regular feature in the protracted cases, and were absent in the less acute cases, in which also films from heart blood were apparently free from organisms.

History. About the middle of March, 1946, a firm of contractors in Calcutta sent to my laboratory two living and one dead duck from their farm. This farm was started about six months previously and to its stock frequent additions were made from time to time from local markets. Prior to the investigation the total number of ducks on the farm was 300. For the previous two months sporadic deaths of one or two ducks were reported, and were ascribed to the overwhelming number of males to females. Little was done to combat these losses, and no serious attention was paid to these isolated reports. This state of affairs continued until suddenly the mortality figure rose to fifteen dead in a single day. The owners, then suspected some contagious disease and segregated the affected birds, but heavy losses continued during the next few days till the matter was referred to me.

Investigation. The duck farm was spacious and was sheltered by tall shady trees. Adjoining was a wide, deep, semicircular pond. Some of the ducks were found to be swimming in the tank, others were resting or moving about with a certain amount of freedom, in a manner that did not seem to prohibit fully indiscriminate mixing.

At the date of my visit, the number of ducks on the farm had diminished from 300 to 150, and about 25 sick birds had been segregated, of which four were already dead. The course of the disease was generally rapid, varying from 16-20 hours, though some took a protracted course and lingered up to three days. The disease appeared to have become widespread and losses at this stage were recorded at the rate of about 30 a day. Six birds (three sick and three recently dead) were brought to the laboratory for further study.

Symptoms. Clinical symptoms were few but were similar and more or less well pronounced in almost every case. There was lameness of both legs, foul-smelling greenish-yellow diarrhoea, and discharge from the eyes, which at times was copious and purulent. In some cases, the eyelids of both eyes were glued together by an adhesive discharge, but on retracting the lids, the eyes seemed to be normal. As the disease progressed the birds lost strength in their legs and rested on their hocks, with head sunken in and turned towards the body. There was respiratory difficulty, marked by a long inspiration and short rapid expirations. Finally, the neck and limbs were stretched and the bird collapsed, the body sometimes turning turtle. There was inappetance and general lethargy throughout, but the leg-weakness was usually the first sign marking the beginning of the malady.

Post-mortem. The carcases were generally in fair condition but heavily infested with lice. On opening the body, the first noticeable feature was an unusual stickiness of the viscera, which were enmeshed in a gelatinous, brownish-yellow exudate. This exudate was so gummy, that it hampered the post-mortem operation. The heart, liver, spleen, lungs, and kidneys showed signs of acute congestion. The intestines were filled with dark-coloured semi-fluid ingesta, and the mucous membrane showed haemorrhagic patches throughout. In two cases the liver was pale and anaemic. Caecal scrapings of two ducks showed fairly frequent trichomonads; from the coecum of another case about two dozen trematodes (*N. attenuatus*) were recovered.

Diagnosis. From the course and the nature of mortality, the contagious nature of the disease was apparent from the beginning. Films of heart blood, liver and spleen from three dead birds at the height of the disease and from two other that had died an hour earlier showed no bacteria. The presence of a large number of living trichomonads from the coecal scrapings of two recently dead birds suggested that these organisms were the cause, but this was not confirmed as no trichomonads were noticed in other cases.

The liver in two other birds which died a little later were enlarged and congested, and showed numerous, minute, discrete, whitish, 'necrotic' foci from pin point to pin head size and sometimes of an irregular shape. The heart fat in the coronary groves showed petechial haemorrhages. Films of heart blood and liver of one of these showed a few *Pasteurella*-like organisms, but from the other bird smears of heart-blood and liver showed in enormous numbers bipolar organism typical of *Pasteurella septica*.

Pathogenicity. About 0·25 c.c. of blood was aspirated into a sterile syringe directly from the heart of the two latterly autopsied ducks and injected subcutaneously into two fowls. Both the fowls died within 23 hours; from them, blood films stained by Leishman method showed large numbers of typical *Past. septica*. Liver smears also showed fairly frequent typical *Past. septica*.

Control. At the time control measures were introduced, only 90 birds were available. These were divided into three groups to be treated with serum, with vaccine and a control group. Unfortunately, the owners insisted that the control should also be treated, so these were given both serum and vaccine. For these purposes, bovine pasteurella serum and vaccine were used, subcutaneously at the rate of 10 c.c. serum and 1 c.c. vaccine for each bird.

Results. The result of vaccination is shown in Table I. It was hoped to keep the vaccinated birds quite separate for careful study, but owing to lack of proper supervision on the part of the owners this plan did not entirely succeed. Post-mortem examination was continued time to time in birds dying after vaccination. *Past. septica* was seen in blood films of the dead birds, in small or large numbers.

TABLE I

Attempted control of Pasteurella outbreak in ducks

No. of ducks treated	Product		Number of deaths during post-inoculation period									
			Day									
			1	2	3	4	5	6	7	8	9–11	Total
30	Pasteurella	Serum	3	1	2	4	1	2	1	0	0	14
30	Pasteurella	Vaccine	3	5	3	5	1	2	2	1	0	23
30	Pasteurella	Serum and Vaccine	3	1	2	2	2	2	1	0	0	11
									TOTAL			48

From Table I it may be seen that the outbreak was under control seven days after inoculation. Nevertheless, control was not successful since more birds died during the immediate post inoculation period. Most of deaths occurred in the vaccine group, next in the serum group, while the sero-vaccine group showed the lowest mortality. The difference however between the serum group and the sero-vaccine group was negligible.

SUMMARY

An outbreak mainly, subacute in type, of pasteurellosis in ducks is described. The disease seemed to have been introduced into the flock by infected ducks. Diagnosis was by microscopical examination of heart-blood but the organisms could only be selected in the blood of birds in which the disease was subacute in type. Control measures adopted late in the outbreak had little success

NUTRITIVE VALUES OF DIFFERENT PARTS OF NAPIER GRASS

By INDUBHUSAN CHATTERJEE, M.Sc. (AGR.), L.AG., MD. ABDUL HYE, M.Sc. and MD. SAYED ALI,
Animal Nutrition Section, Dacca Farm

(Received for publication on 29 May 1947)

(With two text-figures)

NAPIER GRASS (*Pennisetum purpureum*) is a fodder which has been recommended for use by the Bengal Agricultural Department. The chief point in its favour is that it is a prolific grower. In the earlier feeding trials conducted during 1932, it was found to contain about 10 to 16 per cent of dry matter and 90 to 84 per cent of water. Seasonal estimation conducted both in 1932 and later on in 1935-36 with plants of different heights from 1 ft. to 6 ft. has shown a variation which is best illustrated (mean basis) in Table I.

TABLE I

Dry matter of Napier grass at different heights (mean values)

Dimensions	1932			1935-36		
	Leaves Per cent	Stems Per cent	Whole plant Per cent	Leaves Per cent	Stems Per cent	Whole plant Per cent
1 ft.	18·69	10·81	14·64	24·94	15·47	19·28
2 ft.	19·42	10·51	13·95	22·27	15·13	17·83
3 ft.	18·45	11·11	15·15	25·31	16·92	19·80
4 ft.	21·85	12·14	14·99	25·36	20·97	22·59
5 ft.	23·37	13·94	16·71	29·68	25·25	26·72
6 ft.	24·57	15·84	18·15	34·25	26·62	28·84

It will be seen that a large percentage of dry matter is in the leaves. There is however a very large difference between the values of 1932 and of 1935-36. This is partly due to the fact that the 1935-36 crop was ratoon continuing from 1932 and partly that the growth was slower in the later year, due to some extent to a drier condition. The larger percentage of dry matter in the ratoon crop raises the question as to how far such increase contributes towards a corresponding increase and efficiency of the nutrients. This may be partially judged from the percentage of nitrogen as set forth in Table II.

TABLE II

Nitrogen percentage of Napier grass at different heights (mean values on dry basis)

Dimension	NITROGEN 1932			NITROGEN 1935-36		
	Leaves Per cent	Stems Per cent	Whole plant Per cent	Leaves Per cent	Stems Per cent	Whole plant Per cent
1 ft.	1·83	1·23	1·66	1·37	0·81	1·10
2 ft.	1·50	0·94	1·24	1·34	0·68	1·02
3 ft.	1·42	0·77	1·07	1·19	0·47	0·79
4 ft.	1·12	0·52	0·78	0·99	0·36	0·62
5 ft.	1·11	0·42	0·72	0·84	0·25	0·47
6 ft.	1·33	0·43	0·78	0·48	0·33	0·38

Even a cursory glance of Tables I and II will show that as the percentage of dry matter has increased, there has been a progressive fall of the percentage of nitrogen. In other words they seem to vary in an inverse ratio. How far such a variation throws its reflex on the actual yield of both nitrogen and dry matter per unit area is also a matter of great importance. This is illustrated in Figs. 1 and 2.

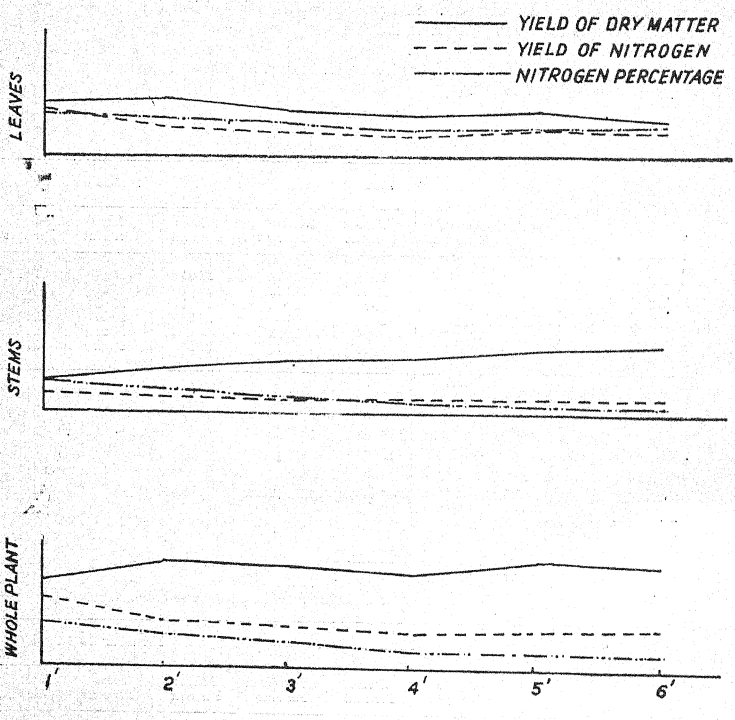

FIG. 1. The Yields of dry matter and nitrogen and the percentage of nitrogen on dry basis of Napier grass of different heights in 1932.

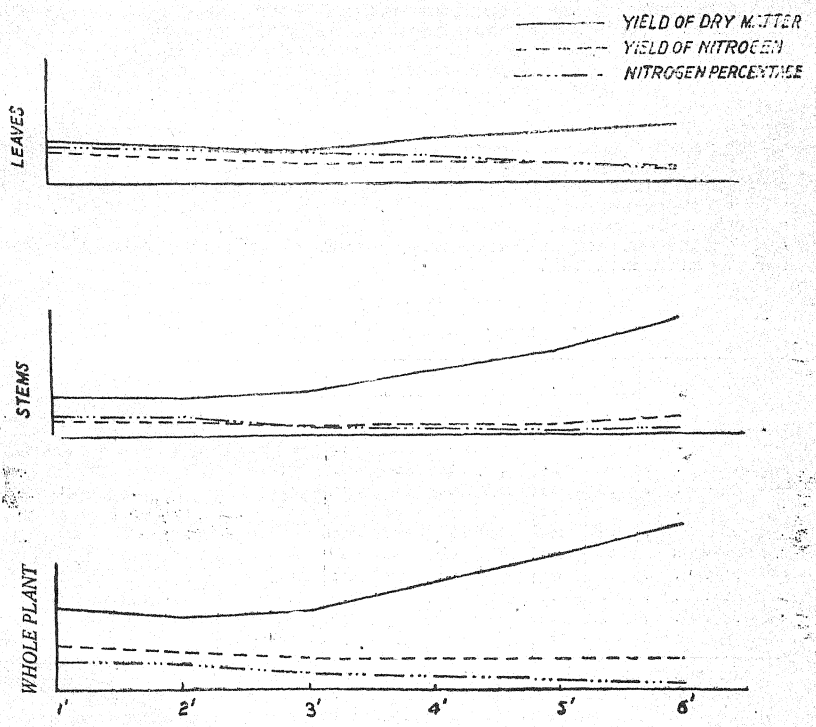

Fig. 2. The Yields of dry matter and nitrogen and the percentage of nitrogen on dry basis of Napier grass of different heights in 1935-1936.

In these graphs (Figs. 1 and 2) the yield of dry matter, the yield of nitrogen and the percentage of nitrogen on dry basis have been plotted against 1 ft., 2 ft., 3 ft., 4 ft., 5 ft., and 6 ft crops for 1932 and 1935-36. Though there has been a large difference between the actual yield for two years, there is broadly much agreement between the bearing of the nitrogen to the dry matter. For instance, if we take the case of the whole plant the yield of nitrogen has been highest at 1 ft. crop, at 2 ft. crop there is a fall which continues up to 3 ft. from which stage it is virtually constant. The dry matter however progressively increases though there is a slight variation for 4 ft. and 6 ft. crop in 1932. There are two points of interest. From the nutritive standpoint, which stage viz. of 3 ft., 4 ft. or upwards provides the best combination of digestible dry matter and digestible protein? From the standpoint of plant physiology it opens out an interesting query as to what becomes of the fate of the extra nitrogen which the 1 ft. plant first imbibes but which is apparently lost as the plant grows in height. From the economic side it is necessary to trap this nitrogen and harness it for increasing a valuable source of nutritive components. This is however a work for the plant physiologist.

If the distribution of nitrogen and dry matter between leaves and stems is ex... the largest amount of nitrogen, whether in terms of total yield or percentage, falls ... leaves. The converse is the case with the dry matter. In this the stem accounts for ... share with the lowest percentage and total yield of nitrogen. Another interesting feature ... up to the 5 ft. stage the yield of dry matter of the leaves does not seem to exhibit significant var... The drop at 6 ft. is largely because of slower growth during the dry season. The results sugg... that the outturn of leaves (dry weight) does not undergo precipitous variation. There is no doubt some fall in the outturn of nitrogen (in leaves) per unit area. The main variation is with the stem which increases its dry matter as it increases in height but at the same time it translocates its nitrogen to the leaves and shows a decreasing amount in the percentage or yield.

Since the criterion of digestibility and assimilability is closely connected with the coarseness and quantity of dry matter specially from the stem, it would seem that growth above 3 ft. (or at the most 4 ft.) is likely to react adversely against economic utilization. Only a limited amount of analysis of the organic components of the composite samples of 1932 crop was conducted and the results are setforth in Table III.

TABLE III

Composition of Napier grass of different heights (dry basis)

Components	Leaves						Stem					
	1 ft.	2 ft.	3 ft.	4 ft.	5 ft.	6 ft.	1 ft.	2 ft.	3 ft.	4 ft.	5 ft.	6 ft.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Crude protein	14·8	8·4	8·8	7·2	6·5	8·1	10·7	6·9	6·5	5·3	3·8	2·7
True protein	12·5	6·3	6·7	5·7	4·3	7·3	6·8	4·9	4·4	4·4	2·5	2·2
Ether extract	2·4	2·6	3·4	3·2	2·3	2·5	2·2	1·4	1·9	1·8	1·0	0·9
Crude fibre	21·8	26·4	30·1	30·7	30·7	31·3	28·9	26·7	29·4	28·8	33·1	32·9
Nitrogen-free extract	44·0	49·9	45·6	48·2	48·8	47·2	41·9	51·7	47·4	51·0	49·8	54·1
Ash	17·1	12·7	12·1	10·8	11·8	11·1	16·4	13·4	14·8	13·2	12·4	9·4
Soluble ash	9·3	7·5	7·0	7·0	..	5·8	12·8	11·0	11·9	11·8	10·1	7·5
Silica	7·8	5·2	5·1	3·8	..	5·3	3·6	2·4	2·9	1·4	2·3	1·9
Chlorine (estimated from ash)	0·1	0·2	0·3	0·2	..	..	0·7	0·6	0·6	0·6	0·6	..

The main feature here is a higher percentage of protein in the 1 ft. stage which can be detected in Figs. 1 and 2 also. From the 2 ft. stage the protein content in the leaves remains about the same but in its stem it has gone down. As can be expected the ether extract percentage is more in the leaves than in the stems. With respect to fibre the lowest percentage (21·78 per cent) has been recorded for leaves at the 1 ft. stage, and to some extent upto the 2 ft. stage, but above heights they are about the same. As a matter of fact from the 3 ft. stage the percentage of crude fibre whether in leaves or stems is about the same. At the same time the fibre fraction of the leaves is likely to be better digested than that of the stem.

The main feature of the ash content is that the stem has more soluble matter and correspondingly less acid insoluble residue than the leaves. This aspect will be taken up again later.

VARIATION IN THE NUTRITVE MAKE-UP OF THE SOFTER AND THE COARSER PARTS

It will be noted from a general survey of the preceding that possibly somewhere about the 3 to 4 ft. stage the combinations of components are such as to provide an economic utilization. An experiment conducted in 1941 suggests this in a more impressive manner. The indications were obtained in the following way. During the course of feeding *amon* straw and Napier grass it was noticed that the animals often left an appreciable fraction of the harder part of the stem. It was

therefore considered advisable to see how the composition of this part varied with the rest. A somewhat arbitrary procedure was adopted.

The Napier grass was fed chaffed but as soon as the grass was brought for chaffing not less than twenty plants were picked at random, the length of each plant was measured and then all the plants weighed. A sort of eye and hand test was used by which, depending on the nature of individual plant, the bottom part of the stem (i.e. the coarser part) was chopped off. The lengths of the two portions, viz. the bottom part as well as the remaining part of the stem, were measured out and both the pieces were immediately weighed. The leaves were also separated, measured and weighed. Proportionate parts after calculation were weighed out first for the dry matter estimation and then to serve as composite samples for chemical analysis. Daily estimations of nitrogen and dry matter of the different fractions were carried out. In Tables IV, V and VI the dimensions of different parts, dry matter and nitrogen are set up.*

* In the feeding trial the coarser part of the stock was removed before the grass was choffed. The animals ate better and left less.

TABLE IV

Dimensions, and percentage of dry matter of different fractions of Napier grass

Date	Leaves	Dimensions			Dry matter			
		Softer stem	Coarser stem	Total	Leaves	Softer stem	Coarser stem	Whole plant
					Per cent	Per cent	Per cent	Per cent
11th July 1941	3 ft. 5 in.	3 ft. 2·5 in.	1 ft. 0 in.	4 ft. 2·5 in.	21·5	12·3	16·1	15·4
12th July 1941	2 ft. 10·35 in.	2 ft. 11·8 in.	0 ft. 5·5 in.	3 ft. 5·3 in.	22·5	14·5	17·6	17·5
13th July 1941	3 ft. 0·5 in.	2 ft. 6·1 in.	0 ft. 9·1 in.	3 ft. 3·2 in.	21·2	13·3	16·1	16·1
14th July 1941	3 ft. 6·15 in.	3 ft. 8·95 in.	0 ft. 11·0 in.	4 ft. 7·95 in.	20·2	12·3	17·1	15·0
15th July 1941	2 ft. 7·2 in.	2 ft. 7·8 in.	0 ft. 8·6 in.	3 ft. 4·4 in.	20·7	13·1	16·5	15·8
16th July 1941	2 ft. 7·8 in.	3 ft. 4 in.	0 ft. 8·7 in.	4 ft. 0·7 in.	22·2	14·1	18·2	17·2
17th July 1941	2 ft. 7·95 in.	2 ft. 6·3 in.	0 ft. 7·1 in.	3 ft. 1·4 in.	23·5	16·1	19·8	18·9
18th July 1941	2 ft. 11·8 in.	2 ft. 11·5 in.	0 ft. 8·25 in.	3 ft. 7·75 in.	22·0	19·0	19·6	20·1
19th July 1941	3 ft. 1·2 in.	2 ft. 4·7 in.	1 ft. 0·5 in.	3 ft. 5·2 in.	21·7	14·3	18·5	17·0
20th July 1941	3 ft. 2·45 in.	3 ft. 5·85 in.	0 ft. 7·3 in.	4 ft. 1·15 in.	21·2	15·5	20·2	17·8
21st July 1941	2 ft. 9·5 in.	3 ft. 3·6 in.	0 ft. 8·6 in.	4 ft. 0·2 in.	21·0	15·1	19·0	17·4
22nd July 1941	2 ft. 9·8 in.	2 ft. 11·1 in.	0 ft. 8·3 in.	3 ft. 7·4 in.	22·8	16·2	20·2	18·8
23rd July 1941	2 ft. 8 in.	2 ft. 9·6 in.	0 ft. 7·6 in.	3 ft. 5·2 in.	23·3	15·6	20·0	18·5
24th July 1941	2 ft. 10·7 in.	3 ft. 2·0 in.	0 ft. 9·4 in.	3 ft. 11·4 in.	23·0	15·2	19·3	18·1
25th July 1941	2 ft. 10·3 in.	2 ft. 8·2 in.	0 ft. 7·7 in.	3 ft. 3·9 in.	24·4	17·9	22·4	20·8
26th July 1941	2 ft. 10·8 in.	3 ft. 0·05 in.	0 ft. 10·1 in.	3 ft. 11·05 in.	25·8	17·5	22·3	20·7
27th July 1941	2 ft. 8·2 in.	2 ft. 11 in.	0 ft. 10·02 in.	3 ft. 9·02 in.	24·6	16·6	21·9	19·9
28th July 1941	2 ft. 11·5 in.	3 ft. 3 in.	0 ft. 11·8 in.	4 ft. 2·8 in.	26·3	19·4	25·6	22·6
29th July 1941	2 ft. 11·1 in.	3 ft. 1·9 in.	0 ft. 11·4 in.	4 ft. 1·3 in.	22·6	18·1	23·2	20·5
30th July 1941	2 ft. 9·3 in.	2 ft. 11·2 in.	0 ft. 10·8 in.	3 ft. 10 in.	25·3	17·6	23·3	21·1
31st July 1941	2 ft. 11 in.	3 ft. 5 in.	1 ft. 0·3 in.	4 ft. 5·3 in.	23·7	16·7	21·7	19·6

The data covered a daily record of 21 days and broadly it may be stated that, on an average, the length of the stem (Table IV) can be taken at 4 ft., whereas the so-called rejected or coarser part was not more than 9 in. on the same basis. In other words the top $3\frac{1}{4}$ ft. of the stem was the softer and possibly the more edible and assimilable part.

If now the percentages and distributions of dry matter and nitrogen are examined, it is noted that the leaves contain the largest percentage of dry matter (20 per cent to 26 per cent), the coarser

TABLE V

Nitrogen percentage of different fractions of Napier grass on green and dry basis

Dates	Nitrogen							
	Fresh basis				Dry basis			
	Leaves	Softer stem	Coarser stem	Whole plant	Leaves	Softer stem	Coarser stem	Whole plant
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
11th July 1941 . .	0·37	0·13	0·06	0·18	1·71	1·06	0·35	1·14
12th July 1941 . .	0·32	0·10	0·05	0·15	1·44	0·66	0·27	0·84
13th July 1941 . .	0·27	0·10	0·04	0·13	1·26	0·75	0·27	0·82
14th July 1941 . .	0·41	0·08	0·07	0·15	2·03	0·63	0·40	1·03
15th July 1941 . .	0·27	0·06	0·04	0·11	1·30	0·45	0·27	0·70
16th July 1941 . .	0·31	0·10	0·06	0·15	1·38	0·73	0·35	0·88
17th July 1941 . .	0·32	0·08	0·04	0·14	1·37	0·49	0·22	0·76
18th July 1941 . .	0·29	0·07	0·07	0·13	1·2	0·38	0·34	0·62
19th July 1941 . .	0·32	0·11	0·06	0·16	1·49	0·74	0·31	0·94
20th July 1941 . .	0·24	0·10	0·05	0·13	1·15	0·61	0·26	0·75
21st July 1941 . .	0·33	0·08	0·06	0·15	1·56	0·52	0·32	0·86
22nd July 1941 . .	0·31	0·10	0·05	0·15	1·38	0·62	0·23	0·81
23rd July 1941 . .	0·32	0·11	0·07	0·16	1·37	0·68	0·36	0·87
24th July 1941 . .	0·29	0·09	0·04	0·14	1·28	0·59	0·22	0·76
25th July 1941 . .	0·30	0·06	0·05	0·13	1·21	0·35	0·24	0·63
26th July 1941 . .	0·27	0·08	0·05	0·13	1·04	0·47	0·21	0·61
27th July 1941 . .	0·27	0·08	0·03	0·12	1·11	0·48	0·15	0·62
28th July 1941 . .	0·35	0·09	0·06	0·16	1·33	0·48	0·24	0·69
29th July 1941 . .	0·25	0·07	0·06	0·12	1·10	0·41	0·25	0·59
30th July 1941 . .	0·30	0·07	0·05	0·14	1·17	0·37	0·22	0·68
31st July 1941 . .	0·30	0·07	0·04	0·12	1·25	0·39	0·17	0·61

TABLE VI

Proportion of components in the different fractions of Napier grass

Dates	Distribution of 100 parts of components in the different fractions								
	Green matter Total=100			Dry matter Total=100			Nitrogen Total=100		
	Leaves	Softer stem	Coarser stem	Leaves	Softer stem	Coarser stem	Leaves	Softer stem	Coarser stem
11th July 1941 .	25·6	54·2	20·2	35·6	43·3	21·1	53·4	40·1	6·4
12th July 1941 .	27·5	48·0	24·5	35·4	39·9	24·7	60·9	31·2	7·9
13th July 1941 .	26·7	49·8	23·5	35·2	41·3	23·5	54·4	37·8	7·8
14th July 1941 .	23·6	58·6	16·8	31·8	47·9	20·3	62·9	29·3	7·9
15th July 1941 .	26·1	54·1	19·8	34·3	45·0	20·7	63·4	28·8	7·8
16th July 1941 .	27·9	51·7	20·4	36·0	42·4	21·6	56·4	35·1	8·5
17th July 1941 .	29·1	52·9	18·1	35·1	45·0	18·9	65·4	29·2	5·4
18th July 1941 .	24·7	58·0	17·3	28·1	54·9	16·9	57·2	33·5	9·3
19th July 1941 .	27·6	57·6	14·8	35·3	48·6	16·2	56·2	38·4	5·4
20th July 1941 .	29·6	56·8	13·7	35·1	49·5	15·4	54·2	40·5	5·3
21st July 1941 .	29·9	54·2	15·9	35·9	46·9	17·2	65·4	28·2	6·4
22nd July 1941 .	28·8	53·3	17·9	34·9	45·9	19·2	59·2	35·2	5·6
23rd July 1941 .	28·3	54·5	17·3	35·5	45·9	18·6	56·4	35·8	7·8
24th July 1941 .	28·3	53·7	18·0	35·8	45·0	19·2	59·9	34·6	5·5
25th July 1941 .	30·7	50·3	19·1	36·1	43·4	20·6	68·7	23·7	7·6
26th July 1941 .	27·0	52·7	20·3	33·6	44·5	21·9	57·8	34·8	7·4
27th July 1941 .	27·5	51·6	20·9	34·0	43·0	23·0	61·2	33·2	5·7
28th July 1941 .	27·0	51·1	21·8	31·4	43·8	24·7	60·7	30·6	8·6
29th July 1941 .	29·1	48·9	22·0	32·1	43·0	24·9	59·6	29·9	10·6
30th July 1941 .	28·6	49·6	21·8	34·3	41·5	24·2	61·4	30·8	7·8
31st July 1941 .	26·8	49·7	23·5	32·4	42·2	25·5	66·0	26·9	7·0

stem is intermediate (16 per cent to 23 per cent), and the softer stem the lowest at 12 per cent to 19 per cent, whereas on the basis of the whole plant the dry matter varies from 15 per cent to 23 per cent (Table IV). If the proportionate distribution of green and dry matters are examined (Table VI), it will be noted that the leaves and softer stems together account for about 80 per cent leaving 20 per cent to the rejected or coarser part of the stem. This is further corroborated by the mean given in Table VII.

If now the nitrogen is examined on a similar basis (Tables V and VI), the highest percentage of nitrogen on a dry basis is in the leaves (1 to 2 per cent), the softer stem is intermediate at 0·4 per cent to 1 per cent and the coarser stem lowest at 0·2 per cent to 0·4 per cent. The differences are still strikingly demonstrated on the basis of their proportionate distribution. Here it will be noted (Table VI) that if the nitrogen present in the whole plant is taken at 100, not more than 5 to 8 parts (with the exception of the three in which it has been between 8·6 to 10·6) are in the coarser stem. In other words over 90 per cent are certainly available in the leaves and softer stem, possibly in a more palatable and assimilable form for the nourishment of the animal. The largest proportion is in the leaves (53 to 69 parts), while the softer stem accounts for 24 to 40 parts.

It is interesting at this stage to compare how the other organic and mineral nutrients vary. As they could not be estimated separately for each day's sample, a composite sample was made on a proportionate basis from the collection of 15 days from 15th July 1941 to 29th July 1941. The data are set up in Table VII.

TABLE VII

Composition and distribution of nitrogen in different fractions of Napier grass

Components	Composite samples from 15th July 1941 to 29th July 1941 (15 days) Percentage of nutrients								Distribution of nutrients per 100 parts		
	Green basis				Dry basis				Total of each = 100		
	84/1941 Leaves	85/1941 Softer stem	86/1941 Coarser stem	Whole plant	84/1941 Leaves	85/1941 Softer stem	86/1941 Coarser stem	Whole plant	Leaves	Softer stem	Coarser stem
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent			
Dry matter	22·95	16·23	20·57	18·91	100·0	100·0	100·0	100·0	34·0	46·0	20·0
Organic matter	20·89	15·11	19·88	17·61	91·03	93·07	96·67	93·11	33·3	46·0	20·7
Total nitrogen	0·29	0·09	0·05	0·14	1·28	0·53	0·26	0·73	59·9	33·1	7·0
Albuminoid nitrogen	0·23	0·05	0·04	0·10	1·00	0·33	0·20	0·53	64·0	28·4	7·6
Crude protein	1·84	0·53	0·33	0·86	8·01	3·28	1·61	4·56	59·9	33·1	7·0
True protein	1·44	0·33	0·26	0·63	6·26	2·06	1·28	3·33	64·0	28·4	7·6
Ether extract	0·64	0·17	0·10	0·29	2·77	1·02	0·47	1·51	62·6	31·2	6·2
Crude fibre	7·23	5·73	7·93	6·56	31·52	35·28	38·56	34·66	30·9	46·8	22·3
Nitrogen-free extract	11·18	8·68	11·52	9·91	48·72	53·49	56·03	52·38	31·6	46·9	21·5
Total carbohydrate	18·42	14·41	19·45	16·46	80·25	88·77	94·59	87·04	31·4	46·9	21·7
Ash	2·06	1·13	0·68	1·31	8·97	6·9	3·33	6·98	44·2	46·1	9·7
Insoluble residue	1·09	0·34	0·26	0·54	4·76	2·13	1·25	2·84	57·0	34·2	8·8
Soluble ash	0·97	0·79	0·42	0·77	4·22	4·81	2·07	4·06	35·3	54·5	10·2
Calcium (CaO)	0·12	0·03	0·03	0·06	0·51	0·22	0·12	0·29	60·1	31·6	8·3
Magnesium (MgO)	0·08	0·08	0·05	0·07	0·37	0·40	0·24	0·39	32·6	55·0	12·4
Potash (K2O)	0·42	0·38	0·14	0·35	1·84	2·36	0·66	1·83	34·2	58·6	7·2
Soda (Na2O)	0·03	0·02	0·03	0·02	0·11	0·13	0·15	0·11	33·0	41·1	25·9
Phosphate (P2O5)	0·14	0·14	0·10	0·13	0·62	0·80	0·48	0·70	30·0	56·4	13·6
Chloride (Cl2)	0·03	0·05	0·02	0·04	0·14	0·36	0·17	0·22	21·8	63·2	15·0

It is noted here that the coarser stem has the largest percentage of organic matter (96·7 per cent), chiefly fibre (38·6 per cent) and nitrogen-free extract (56 per cent); as a matter of fact these constitute such a large part of the organic make-up that very little is left for the other nutritive components specially protein (1·61 per cent) or fat (0·47 per cent); much of the nitrogen-free extract is however embedded in the hard integument which prevent its full assimilation. The coarser stem is also very poor in lime (0·12 per cent), a characteristic of the whole plant (0·29 per cent CaO). The leaves contain the largest percentage of protein (8·01 per cent) and lime (0·51 per cent), while the softer stem stands second in protein content but first in soluble ash (4·82 per cent), potash (2·33 per cent) magnesia (0·46 per cent), phosphorous (0·86 per cent) and chloride (0·31 per cent). Taken on the whole the more useful components exist in larger percentages in the leaves and softer stem.

This aspect is more clearly demonstrated on the basis of proportionate distribution of nutrients shown in the last three columns of Table VII. It will be noted that per 100 parts of whole plants (leaves, softer stem and coarser stem) about 79 parts of organic matter are distributed between the leaves (33·3 parts) and the softer stem (46·0 parts) leaving nearly 21 parts for the coarser stem. Protein and ether extract run parallel, being divided between 6 to 7 parts in the coarser stem, 31 to 33 parts in the softer stem and 60 to 63 parts in the leaves. Though the actual fibre content is highest in the coarser stem, it only forms 22·3 parts for 100 in the whole plant. This is due to the fact that the entire coarser stem does not constitute more than one-fifth of the whole plant.

Of the soluble ash 90 parts are divided between the leaves (35·3 parts) and the stem (54·5 parts) leaving 10·2 parts for the coarser stem. The leaves also contain most of insoluble ash (57 parts) as compared to 34·2 parts in the softer stem and 8·8 parts in the coarser stem.

Of individual minerals the leaves have 60·1 parts of lime, the softer stem 31·6 parts and the coarser stem 8·3 parts.

The softer stem have the largest share of magnesia, potash, phosphate and chlorine, viz. 55·0, 58·6, 56·4 and 63·2 respectively. Contrary to expectation phosphate has been found highest (both in composition and distribution) in the softer stem and less in the leaves.

Taking all facts into consideration the coarser stem, if rejected, will account for a loss of 20 parts organic matter, about 8 to 9 parts of lime, 14 parts of phosphate, 26 parts of soda and 7 parts of potash. The animal is generally inclined to reject much of this part.

The object of this work was to ascertain whether in feeding Napier grass it would be more economical to put the whole plant to the feeding trough or to discard the coarser part. The results suggest that the coarser part is poor in composition with respect to all components except fibre and nitrogen-free extract. But on account of its coarse and tough integuments it is bound to involve an expenditure of a large amount of energy in the work of digestion. It is likely that its exclusion from the diet might prove more economical than its inclusion because of the saving of energy and because the diet would be more efficiently digested the loss of essential minerals would be negligible.

Summary

1. The composition of the coarse stem, the rest of the stem, and the leaves of Napier grass, a 1 ft., 2 ft., 3 ft., 4 ft., 5 ft. and 6 ft. high grown in Bengal was assayed and is discussed.

2. It is suggested that the coarse part of the stem is not worth feeding.

SOME DIGESTIBILITY TRIALS ON INDIAN FEEDING STUFFS

XIII. *DESI* SUGARCANE SACCHRUM BARBARI JESW (*KATHA*)

By LAL CHAND DHARMANI, AGRICULTURAL CHEMIST II, KARTAR SINGH LOHARA, B.Sc., (AGRI.) and N. AKBAR ALI, B.Sc. (AGRI.)

(Received for publication 26 Oct. 1945)

THICK Coimbatore varieties of sugarcane introduced by the Department of Agriculture in the Punjab, yield higher tonnage of cane and bring more profit to the growers than *desi* sugarcane. Inspite of this the zamindars in the Punjab still grow *desi* varieties of sugarcane on a fairly large scale and Katha is one of the most favourite canes. The main reason of its finding favour with the zamindars is that during the days of fodder scarcity this cane can be fed to cattle, being thinner than all the Coimbatore varieties. It has a great tillering capacity and can stand drought, flooding and frost. Its thickness (average diameter) is 1·44 c.m. Stripped cane forms 66 per cent of the whole.

Digestibility trials were conducted with Katha sugarcane on five Sahiwal heifers with a view to find out the feeding value of this cane as compared to wheat *bhusa*, the staple fodder of cattle in the Punjab.

Five Sahiwal heifers Nos. 171, 200, 212, 199 and J. D., two to three years old, were used as experimental animals for the digestibility trials. The technique followed was the same as is usually employed in such trials at the Lyallpur Animal Nutrition Centre [*Memoirs of the Department of Agriculture in India*, 1928, IX (7)]. The experimental animals were kept on the usual preparatory period of 15 days. During the experimental period all the animals were given weighed amount of the feed and their voidings were carefully collected and sampled for detailed analysis in the laboratory.

The entire sugarcane including tops and dry leaves was cut into half inch pieces with a chaff cutter before feeding to the animals. Three digestibility trials were carried out with this feed during the months of December and January.

RESULTS

The chemical composition of the feed employed during the three trials is given in Table I. The chemical composition of wheat *bhusa* is also given for sake of comparison in the same Table. The digestibility data and the nutrients digested from 100 lb. of the feed along with the total digestible nutrients are given in Tables II and III the nitrogen balance is shown in Table IV.

TABLE I

Chemical composition of sugarcane and wheat bhusa

Period	Percentage of dry matter	Percentage of ash	Percentage of fat	Percentage of fibre	Percentage of N.F.E.	Percentage of protein
			Sugarcane			
I	40·65	2·99	0·56	12·96	23·59	0·55
II	43·00	3·68	0·51	13·80	24·38	0·63
III	40·00	3·12	0·45	13·30	22·57	0·56
		Percentage on dry matter				
I	100	7·36	1·38	31·87	58·04	1·35
II	100	8·55	1·18	32·12	56·75	1·46
III	100	7·79	1·12	33·26	56·43	1·40
		Wheat Bhusa				
		(as fed)				
..	92·40	9·43	0·88	38·94	40·96	2·19

TABLE II

Digestibility coefficients

Period	Body weight in lb.	Sugarcane eaten per day in lb.	Dry matter eaten per day in lb.	Dry matter	Digestibility coefficients				Protein
					Ash	Fat	Fibre	N.F.E.	
						Heifer No. 171			
I	779	14·60	5·93	57·42	6·71	61·12	57.96	65·98	Negative
II	721	8·00	3·44	51·00	15·65	43·60	50·96	59·60	Do
III	712	13·75	5·50	52·50	3·55	37·65	55·38	60·20	Do
						Heifer No. 200			
I	757	12·65	5·14	53·74	Negative	59·76	53·12	62·00	Do
II	725	8·00	3·44	52·56	17·35	43·60	53·59	59·60	Do
III	687	12·44	4·98	51·02	12·47	33·57	51·75	58·30	Do
						Heifer No. 212			
I	811	18·50	7·52	49·40	Negative	49·77	44·51	58·55	Do
II	737	10·00	4·30	49·46	13·85	45·95	46·75	59·28	Do
III	724	16·31	6·52	51·32	13·40	36·91	51·20	58·47	Do
						Heifer No. 199			
I	675	11·90	4·84	51·25	Negative	55·48	53·67	60·98	Do
						Heifer No. J. D			
I	421	9·91	4·03	54·09	Negative	55·07	55·63	63·63	Do
						Wheat Bhusa			
..	500 to 700	6·00	5·54	48·71	Negative	35·55	61·15	52·51	Do

The results are self explanatory. Digestibility coefficients of different constituents are, more or less, of the same order as those of wheat *bhusa*. The nitrogen balance, which was always negative for all the animals during the three periods, shows that sugarcane when fed alone is a non-maintenance ration.

TABLE III

Digestible nutrients per 100 lb. of feed

Period	Dry matter	Ash	Fat	Fibre	Nitrogen free extract	Protein	Total Digestible nutrients per 100 lb. of feed
				Heifer No. 171			
I	23·32	0·20	0·34	7·51	15·55	negative	
II	21·33	0·58	0·22	7·04	14·54	,,	23·74
III	20·75	0·11	0·17	7·28	13·42	,,	22·02
						,,	21·03
				Heifer No. 200			
I	21·84	..	0·34	6·88	14·60	,,	
II	22·60	0·64	0·22	7·40	14·81	,,	22·18
III	19·84	0·36	0·15	6·67	12·90	,,	22·65
							19·77

TABLE III—*contd.*

Digestable nutrients per 100 lb. of feed

Period.	Dry matter	Ash	Fat	Fibre	Nitrogen free extract	Protein	Total Digestable nutrients per 100 lb. of feed
			Heifer No. 212			negative	
I	20·08	..	0·28	5·77	13·81	,,	20·14
II	20·00	0·51	0·23	6·48	14·46	,,	21·38
III	20·15	0·41	0·16	6·70	13·19	,,	20·21
			Heifer No. 199				
I	20·86	..	0·31	6·99	14·41	,,	20·14
			Heifer No. J. D.				
I	22·48	..	0·32	7·37	15·36	,,	23·37
			Wheat bhusa				
I	44·80	..	0·12	23·80	21·40	,,	45·47

TABLE IV

Nitrogen balance

Period	In-take of nitrogen (gm.)	Nitrogen voided			Balance (gm.)
		In dung (gm.)	In urine (gm.)	Total (gm.)	
			Heifer No. 171		
I	5·84	8·67	8·51	17·18	—11·37
II	2·77	6·16	11·97	18·13	—15·36
III	5·52	7·70	7·53	15·23	—9·71
			Heifer No. 200		
I	5·04	7·33	7·38	14·94	—9·90
II	2·77	6·22	9·86	16·08	—13·31
III	4·92	6·49	7·42	13·91	—9·00
			Heifer No. 212		
I	7·35	8·96	9·49	18·45	—11·10
II	4·37	7·18	10·71	17·89	—13·62
III	6·51	10·12	8·52	18·64	—12·13
			Heifer No. 199		
I	4·95	7·33	7·38	14·71	—9·90
			Heifer J. D.		
I	4·04	6·25	4·42	10·67	—6·63

Katha sugarcane is usually fed as a substitute for wheat *bhusa* during fodder scarcity. It has, however turned out to be inferior to wheat *bhusa* as it is one and a half times richer in protein than sugarcane though total digestible nutrients in both the cases are almost the same. No doubt wheat *bhusa* contains higher percentage of fibre, but its digestibility is higher than that of sugarcane (Wheat *bhusa* contains 25·0 per cent digestible crude fibre while sugarcane contains 16·7 per cent only on dry matter basis). In order to bring up wheat *bhusa* and sugarcane to a maintenance level, heifers No. 199 and J. D. were given wheat *bhusa ad lib.* and 42 gm. of nitrogen in *toria* cake and heifers 171,200 and 212 were fed 45 gm. of nitrogen in *toria* cake with sugarcane given *ad lib.* With almost equal amounts of added nitrogen in their daily ration the heifers fed on wheat *bhusa* showed a positive daily nitrogen balance of 14 gm., whilst the group fed on sugarcane showed a positive daily nitrogen balance of 4·5 gm. only (Table V, which indicates the superiority of the protein in wheat *bhusa*.

TABLE V

Daily nitrogen balance

Heifer No.	Nitrogen in-take			Voided		Nitrogen balance
	In wheat *bhusa* (gm.)	In cake (gm.)	Total (gm.)	In dung (gm.)	In urine (gm.)	(gm.)
			Wheat bhusa-*cake*			
199	6·64	42·0	48·64	18·90	21·10	8·64
J. D.	9·64	42·0	51·64	17·75	14·55	19·34
						Average 13·99
	(*In sugar cane*)		*Sugarcane-cake*			
171	4·5	45·1	49·6	15·3	31·2	3·1
200	4·4	45·1	49·5	12·5	28·5	8·5
212	6·3	45·1	51·4	19·6	30·04	1·8
						Average 4·5

SUMMARY

Digestibility coefficients of different constituents of sugarcane (Katha *desi* variety) were determined with five Sahiwal heifers.

Sugarcane contains 19·8 lb. to 23·8 lb. total digestible nutrients per 100 lb. of the feed. When fed alone it forms a non-maintenance ration. It is inferior to wheat *bhusa* as a cattle feed.

SHELL SEALING OF EGGS

By T. S. KRISHNAN, Poultry Research Section, Indian Veterinary Research Institute, Izatnagar

(Received for publication on 16 June 1947)
(With one text-figure)

THE efficiency of lime and water-glass for egg preservation under Indian conditions was studied by Macdonald and Krishnan [1943]. The preservative action of these substancer is generally attributed to the sealing of the egg shell pores ; which ensues during the storage in their solution. An attempt has been made to determine the minimum time required to effect this.

An increase in size of the egg air-cell is due to evaporation of water from the egg contents, which, other factors remaining the same, depends on the porosity of the egg-shell. In this work it has been assumed that the degree of shell-sealing is inversely proportional to the increase in air-cell depth, i.e., the more complete the sealing, the smaller the increase in air-cell size and vice versa.

Day-old infertile eggs were soaked for varying periods in lime-water and water-glass, prepared as usual [Macdonald and Krishnan, 1943], and then put into an incubator at 100°F. along with an equal number of similar but untreated eggs. After seven days storage, lots of twelve eggs were taken from each of the above series and the depth of the air-cell in each egg was measured (see Table I).

TABLE I

Average air-cell depth in eggs, treated in different ways, after seven days storage at 100°F.

Period of immersion in preserving liquid	Depth of air cell (mm.)		
	Lime-water treated	Water-glass treated	Untreated
6 hours	6·5	11·3	10·9
12 hours	5·8	10·2	11·0
18 hours	5·2	10·5	10·8
24 hours	5·3	11·2	11·2
48 hours	5·1	10·0	11·2
72 hours	5·3	9·6	10·9
96 hours	5·1	8·6	11·0
120 hours	5·1	9·0	11·2

N.B.—The initial depth of air-cell in fresh day-old eggs was 4·5 mm.

The untreated eggs showed an average increase of 6·5 mm. in air-cell depth after seven days at 100°F. Under similar conditions of storage, the increase in air-cell depth in eggs soaked in water-glass for five days was 4·5 mm., as compared with 2·0 mm., 1·3 mm., and 0·7 mm. observed in eggs immersed in lime-water for 6, 12 and 18 hours respectively. Even after 120 hours soaking in lime-water the increase in air-cell size was almost the same as after 18 hours immersion. Hence it seems that the sealing action of lime-water was nearly complete in about 18 hours. Treatment for only six hours with lime-water would appear to be much more effective than five days with water-glass. Even after nine days immersion in water-glass the sealing was only partial as indicated by an increase of 3·5 mm. in air-cell depth. It would therefore appear that the minimum time required for sealing the shell pores in eggs steeped in lime-water is about 18 hours. The action of water-glass seems to be comparatively very slow since only partial sealing resulted even after nine days treatment.

In the case of lime-water the sealing is perhaps due to the deposition in the shell-pores of insoluble $CaCO_3$ formed by the interaction of $Ca(OH)_2$ of the lime-water and CO_2 present inside the egg. Since this chemical action is completed quickly, shell-sealing in lime-water takes place rapidly. This.

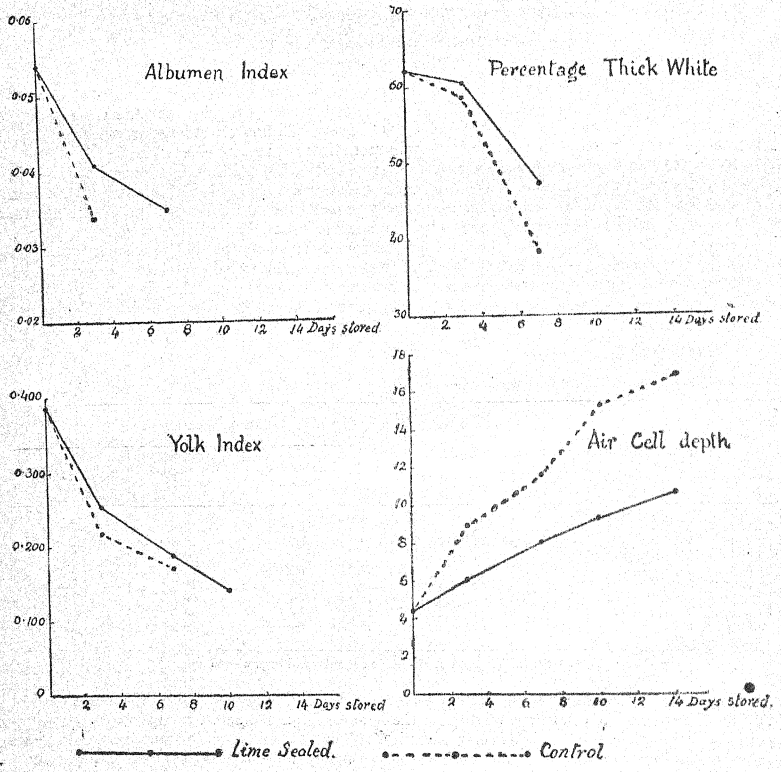

Fig. 1. Interior quality of eggs after storage

view is further supported by the fact that the sealing is quicker and more complete in fresh eggs, which generally contain more CO_2, than in old ones, which usually contain much less of this gas.

With water-glass the sealing is probably due to the deposition in the pores of insoluble gelatinous SiO_2 formed by the hydrolytic dissociation of the Na_2SiO_3 and its consolidation with time. This, as has been observed, is evidently a slow process.

To determine the effect of shell-sealing on the keeping quality of eggs, a comparative study of the interior quality of similar lots of treated and untreated infertile eggs stored at 100°F. (summer conditions) was made. The treatment given was immersion for 12 hours in lime-water. Water-glass was abandoned as being too slow for practical commercial application. The results of the study are presented in Fig. 1. It was found in each test that the interior quality, as judged by albumen index (Average diameter *Height* of thick white), percentage thick white and yolk index (*Height*/Diameter of yolk), was somewhat better in the treated eggs than in the untreated ones. The air-cell depth, however, showed a marked difference in the two lots. The treated ones had a significantly smaller air-cell depth than the controls at all stages of the storage period. In the above experiment, which simulates summer conditions, the maximum allowable air-cell depth of 9·5 mm. ($\frac{3}{8}$ in.) according to the Agricultural Grading and Marking Act, was attained in the untreated eggs in four days, while in the treated ones this level was reached only after 11 days. In the commercial grading of eggs by candling, considerable weight is attached to air-cell size. Those with air-cells of more than $\frac{3}{8}$ in. depth are rejected, below this limit eggs with bigger air-cells are relegated to lower grades. In the hot summer months, when temperatures are high and humidity low, evaporation from eggs is rapid and consequently air-cells increase in size quickly. Since the bulk of the eggs are produced in villages and consumed in towns and cities, they have to be transported from the rural areas to the urban and pass through various trade channels in the process. Consequently they take a few days to reach the consumer. During the hot weather, unless eggs reach the consumer within about 3-4 days of their being laid, for reasons already stated they may be rejected as under-grade, resulting in serious losses to the trade. If, however, soon after collection their shells are sealed by immersion in lime-water for even 12 hours, their quality would be maintained better despite these adverse conditions, for about a week, thereby giving more time for marketing them and thus avoiding loss and wastage, besides providing the consumers with a better quality product. This method would also keep down shrinkage and conserve the quality of eggs transported over long distances during summer months.

In the United States of America and other western countries shell-sealing of eggs is generally carried out by dipping in oil. The cost of the oil, which is patented, the difficulty of obtaining it and the greasiness of shell resulting from this treatment are all handicaps in its adoption to Indian conditions. With lime-water, on the other hand, the cost is negligible, the material is readily available everywhere, its method of preparation and use are simple and require little skill, the period of treatment is reasonably short and its use results in substantial benefits to the egg trade. It may therefore be recommended for general adoption by village producers and traders who handle only small numbers of eggs at a time. They are advised to put the eggs in lime-water for at least 12-18 hours, as quickly as possible after they are laid, and store them in the coolest place available. The eggs should be removed from the liquid only at the time of sale. Better results are obtained if the eggs are infertile or defertilized [Krishnan and Macdonald, in the press] before the lime treatment. The process is intended only for use with table eggs.

SUMMARY

The comparative efficiency of lime-water and water-glass for egg preservation under Indian conditions has been studied. Treatment for only six hours with lime water appeared to be much more effective than five days with water-glass. Immersion for 18 hours in lime water seems to be sufficient for shell sealing.

REFERENCE

Macdonald and Krishnan (1943). *Indian J. vet. Sci.*, **13**, 306

SELECTED ARTICLE

EFFECT OF LOCAL INJECTIONS OF PENICILLIN ON STAPHYLOCOCCI IN THE COW'S UDDER*

By Louis A. Klein, V.M.D., David W. Crisman, B.S., D.V.M., and John W. Moor, V.M.D.,
Philadelphia, Pennsylvania

THE results obtained with local injections of penicillin in the treatment of empyema, mastoiditis and chronic wound sinuses in man due to staphylococcus or streptococcus infection, as reported by the Floreys,[1] the National Research Council's Committee on Chemotherapeutic and Other Agents,[2] and by Lyons,[3] suggest that intramammary injections via the teat canal might be effective in catarrhal mastitis, also called chronic mastitis.

In this form of mastitis, conditions exist that have been found favourable to the action of local injections in treating human patients, viz., the infection is situated in a closed cavity or space from which the contents can be readily withdrawn, and the infection is localized and accessible. Frei[4] and other investigators have found that in catarrhal mastitis due to streptococci, it is the excretory channels that are involved, usually the milk cistern and the larger ducts primarily, while clinical symptoms indicate that the disease is similarly situated when it is caused by staphylococci. In this location the causative organisms are readily accessible to solutions introduced through the teat canal while any exudate present can be readily milked out before making an injection. Most clinicians with experience in treating human patients with the drug are of the opinion that local injections should be supplemented by intravenous or intramuscular injections but recovery or improvement has been obtained in the conditions mentioned above with local injections alone.

It was decided to test the action of intramammary injections of penicillin on the staphylococci which cause mastitis and also to determine the effect on the cow, especially on the under tissue. The penicillin used was prepared specifically for this study by Dr Waltee Kocholaty, Thos. H. Dougherty, Jr. Fellow in Research in Brucellosis, University of Pennsylvania School of Medicine, in July, 1943.

Staphylococci are one of the causes of catarrhal mastitis and the symptoms they produce are similar to those caused by streptococci. They also cause a severe type of acute perenchymatous mastitis which is often complicated by sepsis, and gangrene of the udder is frequently associated with it. This type of udder infection was selected because clinical results show that it is more resistant to the agents now used in intramammary injections than the mastitis streptococci, and also because in *in vitro* tests penicillin was found to be more effective than some of these agents against *Staphylococcus aureus*. Fleming[5] found it four times as potent as sulfathiazole and twenty times as potent as sulfapyridine against this organism, and Heilman and Herrell[6] found it more effective than gramicidin.

Identification of mastitis staphylococci

In examining samples of milk or other udder secretions for the staphylococci which cause mastitis, it is necessary to differentiate them from the nonpathogenic cocci, usually called micrococci, which are normally present in the udder and the milk.

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* From the School of Veterinary Medicine, University of Pennsylvania, Philadelphia.

In accordance with regulations formulated by the War Production Board, concerning the production and use of penicillin, permission was requested and received by Dr G. A. Dick, Dean of the School of Veterinary Medicine, to produce and use a limited amount of penicillin for the purpose of this study. At the request of Dr A. N. Richards, Vice President of the University of Pennsylvania and Chairman of the Committee on Medical Research of the Office of Scientific Research and Development, publication of the findings of this study has been withheld until the present. The contents of this paper were, however, submitted in confidence on April, 29, 1944, to the Bureau of Animal Industry, U. S. Department of Agriculture, and to the Pennsylvania Department of Agriculture.

Publication of this article has been cleared through the Committee on Medical Research, National Research Council.

This study was facilitated by laboratories of the University of Pennsylvania, which receive financial support from the U. S. Department of Agriculture, the Department of Agriculture of the Commonwealth of Pennsylvania, the Smith, Kline and Fresh Laboratories Inc., and the Thos. H. Dougherty Jr. Fund.

Minett[7] found that hemolysis on ox-blood or sheep-blood agar and coagulation of human or rabbit-blood plasma were characteristic of staphylococci isolated from milk samples, from udder quarters affected with chronic mastitis, and from the affected quarters of cows showing symptoms of acute, septic parenchymatous mastitis, and that it is improbable that cocci recovered from samples of milk from normal quarters, which do not possess these properties, are pathogenic. Plastridge and his associates[8] examined staphylococci isolated from milk samples from individual quarters of milking cows for their ability to hemolyze ox-blood and coagulate human-blood plasma, for pigment formation, leucocyte count, and other properties, using the leucocyte count as an index of pathogenicity.

TABLE I

Schedule followed in taking milk samples and making injections of penicillin

Time	Quarters received 8 injections at 6 hour intervals	Quarters received 4 injections at 12 hour intervals
First day	Took Sample 1	Took Sample 1
3 p.m.	Milked out and injected quarter	Milked out and injected quarter
9 p.m.	Milked out and injected quarter	....
Second day	Took Sample 2	Took Sample 2
3 a.m.	Milked out and injected quarter	Milked out and injected quarter
9 a.m.	Milked out and injected quarter	....
3 p.m.	Took Sample 3	Took Sample 3
	Milked out and injected quarter	Milked out and injected quarter
9 p.m.	Milked out and injected quarter	....
Third day	Took Sample 4	Took Sample 4
3 a.m.	Milked out and injected quarter	Milked out and injected quarter
9 a.m.	Milked out and injected quarter	....
3 p.m.	Took Sample 5	Took Sample 5

Took Samples 6, 7, 8, and 9 on the third, sixth, tenth, and thirteenth days following, immediately before the evening milking.

From the results obtained, they concluded that ability to coagulate rabbit or human-blood plasma is more closely related to pathogenicity than any other property. The greater number of the coaguse positive strains were hemolytic but some were not. They also concluded that staphylococci from the udder which are hemolytic on ox-blood agar are usually pathogenic whether they have coagulative ability or not, as are also staphylococci associated with a leucocyte count of 500,000 or over provided the cow is not in the early or late stage of lactation. They found no definite correlation between pigment formation and the leucocyte count. Nearly as many strains of staphylococci associated with a low leucocyte count produced pigmented colonies as those associated with a high count. Chapman, Berens, Peters, and Curcio,[9] who studied staphylococci of human origin and determined pathogenicity by injecting cultures into rabbits, found that while strains which coagulated human-blood plasma were usually pathogenic regardless of the color of the colonies, those which produced hemolytic yellow colonies were usually pathogenic regardless of coagulative ability and hemolytic noncoagulating strains that produced white colonies were usually nonpathogenic.

Plan of experiment

In selecting the cows for this experiment, ability to hemolyze ox-blood, coagulate human-blood plasma, and produce pigment were used, together with the leucocyte count, to differentiate pathogenic staphylococci from the nonpathogenic cocci (micrococci) which are commonly present in cow's milk. The methods by which these determinations were made are described below.

Samples of milk were taken from each quarter of the udder at the time of the evening milking but before the cow was milked. After the udder had been prepared in the usual way to prevent external contamination, three streams were expressed from the quarter and discarded, and 15 to 20 c.c. of milk were then drawn into a sterile container, which was placed in a refrigerator until removed to the laboratory.

TABLE II

Results of examination of milk samples collected immediately before the first injection of penicillin

Cow	Quarter	Staphylococci per c.c.	Pigmentation	Hemolysis	Coagulation	Leucocytes per c.c.
91	LH	80	Medium	Hemolytic	—	1,200,000
10	LF	125	Orange	Hemolytic	+	60,000
10	RH	5400	Light	Hemolytic	+	900,000
1	RH	470	Light	Nonhemolytic	+	1,200,000
27	RF	175	Medium	Hemolytic	+	120,000
216	RF	510	Medium	Hemolytic	—	90,000
10	RF	5100	Light	Hemolytic	+	300,000

Plating. Blood-agar plate were prepared by adding 5·0 per cent of defibrinated cow's blood to melted standard nutrient agar containing 0·5 per cent of sodium chloride and inoculating two plates from each milk samples, one with 1·0 c.c. and one with 0·1 c.c. After 48 hours incubation at 37° C., the plates were examined for hemolytic colonies and for nonhemolytic colonies showing pigmentation. Two plates of standard nutrient agar were inoculated with the same quantities of each sample and examined after 48 hours incubation, since pigmentation could be more readily detected on this medium. Hemolytic colonies of staphylococci and those showing any degree of pigmentation were picked off and streaked on standard nutrient agar. A film was also prepared from one of these colonies and gram-stained for microscopic examination. After the ox-blood agar and standard nutrient-agar plates were prepared, each milk sample was incubated for 24 hours and then streaked on ox-blood agar. These plates were examined after 48 hours incubation at 37 C.

Coagulase test. The technique used by Chapman, Berens, Peters, and Curcio,[9] as modified by Plastridge and associates,[8] was used to determine ability to coagulate human-blood plasma. (Rabbit-blood plasma is equally satisfactory.) A 4 m.m. loopful of a 24-hour-old culture on nutrient agar was mixed with 0·5 c.c. of fresh plasma contained in a $\frac{3}{8}$ in. by 3 in. test tube, held at room temperature, and examined after one, two, three, and twenty-four hours, for evidence of clot formation. Evidence of clotting during the period of observation is a positive result. The human-blood plasma was obtained by drawing 75 c.c. of blood in a flask containing 7·5 c.c. of a 10 per cent solution of sodium citrate, and centrifuging to separate the plasma from the blood cells. The plasma should be used within forty-eight hours.

Leucocyte Count. The slides for the leucocyte count were prepared and the count determined as specified for the direct microscopic method of counting bacteria in milk in *Standard Methods for the Examination of Dairy Products*, 8th edition, pp. 44 to 54.

On the information obtained by the methods of examination described previously, seven quarters were selected for treatment with penicillin—three in 1 cow (10) and one each in 4 others (91, 1, 27, and 216). None of the cows showed any clinical symptoms of mastitis. According to the history obtainable, all had previously had attacks of catarrhal mastitis due to streptococci but none recently. On palpation of the selected quarters, indurated area were found in four, viz. to a 2 in. by 4 in. area in the left front quarter of cow 10 and a similar area in the right hind quarter; a nodular area $2\frac{1}{2}$ in. in diameter in the right hind quarter of cow,[1] with thickening of a part of the mucous membrane of the milk cistern, and an area $2\frac{1}{2}$ in. in diameter in the right front quarter of cow 27.

Schedule of dosage and sampling

Penicillin was used in the form of the sodium salt. This material, at the time of its preparation, assayed 320 Oxford units per milligram. It was contained in sealed glass vials, 120 mg. in each vial, and the vials were closed with rubber stoppers. Difficulty was encountered in making other

arrangements for the study so the penicillin was stored in a refrigerator until its use nine months later, at which time, the assay value was 190 Oxford units per milligram. The contents of a single vial was dissolved in 500 c.c. of sterile normal saline solution and injected in 1 dose. Each dose, therefore, contained 22,800 Oxford units of penicillin. The solution was prepared immediately before injection, the vials being stored in a refrigerator until that time.

TABLE III

Results of examination for staphylococci of milk samples from udder quarters receiving eight injections at intervals of six hours

Sample	Cow 91 L. H. Quarter			Cow 10 L. F. Quarter			Cow 10 R. H. Quarter			Cow 1 R .H. Quarter		
	Staphylococci			Staphylococci			Staphylococci			Staphylococci		
	Poured Plate	Streaked Plate	Coagulation	Poured Plate	Streaked Plate	Coagulation	Poured Plate	Streaked Plate	Coagulation	Poured Plate	Streaked Plate	Coagulation
Before injection—												
1	H	H	—	H	H	+	H	H	+	NH	NH	+
After injection—												
2	—	—		—	—		—	—		—	—	
3	—	—		—	—		—	—		—	—	
4	—	—		—	—		—	—		—	—	
5	—	—		—	—		—	—		—	—	
6	—	—		—	—		H	H	+	NH	NH	—
7	—	—		—	H	+	H	H	+	—	NH	—
8	—	—		—	NH	—	H	H	+	—	NH	—
9	—	—		—	NH	—	H	H	+	—	NH	—

Ox-blood agar plates: H=hemolytic colonies; NH=nonhemolytic colonies; —=no staphylococci colonies; +=coagulation; —=no coagulation.

Four of the quarters selected for treatment were given 8 injections of 500 c.c. of the solution, via. the teat canal, at intervals of six hours, and three received 4 injections, at 12 hour intervals. The treatment was begun at the time of the regular evening milking. Just before the cow was milked, a sample of milk was taken in the manner described previously, from the quarter to be injected. After the cow was milked by the regular milker, the quarter was stripped; the teat wiped with 70 per cent alcohol; and the first injection made. These samples were given the designation 1, and the containers were marked with this number and the number of the cow. The quarter was also indicated. Before each subsequent injection, the quarter to be injected was milked out as thoroughly as possible. Samples were taken every 12 hours from both groups of quarters, i.e. from those receiving 8 injections and those receiving 4 injections. All cows in the experiment were milked before any of the other cows in herd.

The schedule followed in making the injections and taking the milk samples for laboratory examination is presented in Table I. Referring to this Table, it will be seen that the samples taken from the quarters receiving 8 injections and numbered 2, 3, 4 and 5 were drawn from the treated quarters six hours after the last of 2 injections, given six hours apart, and that the samples from the cows receiving four injections and numbered 2, 3, 4 and 5 were each taken 12 hours after a single injection. Samples were also taken from both groups on the third, sixth, tenth, and thirteenth days following the last injection and numbered 6, 7, 8 and 9, respectively. These samples were taken immediately before the evening milking. Each sample container was marked to indicate the number of the sample, the number of the cow, and the quarter.

The results of the examination of samples 1, which were taken immediately before the first injection, are shown in Table II. In samples taken previously from the left hind quarter of cow 91 and from the right front quarter of cow 27, the plate count of staphylococci was much higher than in samples 1, being 11,000 and 14,000 per c.c., respectively. A great variation in the number of staphylococci in samples from the same source, even when they are collected at short intervals, has been reported by a number of investigators.

TABLE IV

Results of examination for staphylococci of milk samples from udder quarters receiving four injection

Sample	Cow 27 R. F. Quarter			Cow 216 R. F. Quarter			Cow 10 R. F. Quarter		
	Staphylococci			Staphylococci			Staphylococci		
	Poured Plates	Streaked Plates	Coagulation	Poured Plates	Streaked Plates	Coagulation	Poured Plates	Streaked Plates	Coagulation
Before injection—									
1	H	H	+	H	H	—	H	H	+
After injection—									
2	—	—		—	—		—	—	
3	—	—		—	—		—	—	
4	—	—		—	—		—	—	
5	—	—		—	—		—	—	
6	—	H	+	—	—		—	NH	+
7	—	H	+	—	H	—	—	H	+
8	—	H	+	H	H	—	—	NH	—
9	—	H	+	—	—		—	H	+

Ox-blood agar plates: H=hemolytic colonies; NH=nonhemolytic colonies; —=no staphylococci colonies; +=coagulation, —=no coagulation.

DISCUSSION OF RESULTS

The results of the examination for staphylococci of the samples collected after the injection of penicillin was begun and in the period following the last injection from the quarters receiving 8 injections are given in Table III. Similar information regarding the samples from the quarters receiving 4 injections is presented in Table IV. On examination of these Tables, it will be seen that staphylococci did not appear on any of the plates inoculated with the milk samples numbered 2, 3, 4 and 5, from either group. Those from the quarters included in Table III were taken after 2 injections of penicillin had been given, the last six hours before, while those in Table IV had received 1 injection twelve hours previously. These results indicate that the antibacterial action of the penicillin continued for 12 hours and that up to this stage of the experiment, 4 injections at 12 hour intervals were as effective as 8 at six hour intervals.

The treated quarter of cow 91 remained free of the infection during the entire period the examination of milk samples was continued, i.e. for 13 days after the last injection. It is probable that the quarter treated in cow 1 also remained free of the original infection since the nonhemolytic strain of staphylococci in samples 6, 7, 8 and 9 from the quarter did not coagulate human-blood plasma as did the nonhemolytic strain which infected this quarter prior to the injection of penicillin. Both these quarters received 8 injections at intervals of six hours. The two other quarters given the

same treatment and also the three that received 4 injections at intervals of 12 hours were apparently free of the injection when samples 5 were taken six and 12 hours after the last injection, but samples 6 or 7, taken on the third and sixth day following the last injection and samples taken subsequently were found to be infected. Since one of the quarters receiving 8 injections was apparently cleared of the infection and another was probably cleared, while the infection disappeared only temporarily from the quarters receiving 4 injections, it would appear that a more prolonged antibacterial action was produced by 8 injections than by 4, although given within the same period of time. However, since no growth of staphylococci occurred on any of the plates inoculated with samples 2 to 5, inclusive, it is possible that they contained sufficient penicillin to inhibit growth of the organism and that growth occurred on the plates inoculated with samples taken subsequently because all of the penicillin injected had been absorbed or eliminated.

Referring to Table IV, it will be noted that sample 6 from the right front quarter of cow 10 was infected with coagulase positive nonhemolytic staphylococci but that the staphylococci found in samples 7 and 9 were hemolytic. A similar variation was observed in milk samples examined before the injections were made. Coagulase positive nonhemolytic staphylococci were present in a sample taken from this quarter 12 days before sample 1.

The first $\frac{1}{4}$ to $\frac{1}{2}$ of the milk obtained from three of the quarters receiving injections at six-hour intervals contained slugs or strings of mucus and was watery after the first injection and the milk of the other quarter receiving the same treatment was similarly affected after the second injection. Later, this portion of the milking was of a greenish or yellowish color. This condition continued up to the time of taking samples 5, six hours after the last injection.

Similar changes occurred in the milk of the quarters receiving 4 injections at eight-hour intervals but to a lesser degree. The milk of one quarter in this group was altered in appearance after the first injection and of the other two after the second injection. The milk of one of the latter was of normal appearance at all other succeeding examinations.

When samples 6 were taken on the third day following the last injection, the milk of all quarters was of normal appearance.

The average daily milk production for one week before and for the first, second, and third weeks after treatment are shown in the following Table :—

Cow	Before treatment	1st Week after	2nd Week after	3rd Week after	Stage of lactation when treated
	lb.	lb.	lb.	lb.	
91	17·15	16·60	16·80	16·10	50th week
10	23·12	21·41	20·03	19·70	38th ,,
1	31·94	29·10	29·90	29·90	15th ,,
27	32·94	35·02	28·80	32·30	11th ,,
216	41·62	38·41	28·80	32·00	14th ,,

Cow 10 had three quarters injected, two with 8 doses and one with 4 doses. One quarter was treated in each of the others. Cows 91 and 1 received 8 doses and 27 and 216, 4 doses.

As to the effect of the injections on the udder tissues, only 1 cow, 91, showed any symptoms of irritation. The lower portion of the quarter treated in this cow was swollen and hardened three hours after the first injection but the swelling was not hot or painful to pressure. In a few hours, the entire quarter was involved and after the second injection the swelling increased slightly. No further change occurred until after the fourth injection. The swelling had begun to decrease and had disappeared entirely on the third day following the last dose.

The temperature of the cows was taken at the time of each injection and was within the normal range in all instances except at the time of the second injection, when it was 103·2 F. in cow 91, 103·2 F. in cow 10, and 103·6 F. in cow 27, and at the time of the third injection 102 F. in cow 216.

SUMMARY

Seven udder quarters, infected with staphylococci, with characteristics corresponding to those of mastitis staphylococci, were given injections via the teat canal of a solution of penicillin in sterile, normal saline solution. Four quarters were given 8 injections of 500 c.c. each at intervals of six hours and 3 received 4 injections at intervals of 12 hours. Each dose contained 22,800 Oxford units of penicillin.

Samples of milk were taken for laboratory examination immediately before the first injection, at intervals of 12 hours during the injection period, and on the third sixth, tenth, and thirteenth days following.

Laboratory examinations indicated that the samples from all of the quarters taken after the first injection were free of the staphylococcus infection. The samples taken subsequently, up to six and twelve hours after the last injection, were also free, but infection again developed on the plates inoculated with samples taken from five quarters on the third or sixth day after the last injection. The original staphylococcus infection has apparently been destroyed in the two other quarters. Both of these quarters received 8 injections.

The results indicate that the antibacterial action continued for twelve hours but 4 doses were not sufficient to obtain a permanent effect.

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REVIEW

The Scotch Shorthorn—The herds of Scotland, England and Wales, 1945-46

By T. B. MARSON (*Published by Macmillan & Co., Ltd., London. Price 2s.*)

THE Scottish Shorthorn Breeders' Association has published a description of every herd of registered Scottish Shorthorns of any size in the United Kingdom so that intending purchasers may conveniently survey the whole field of potential operations.

The compilation was entrusted to Wing Commander Marsden and he has succeeded in exhibiting the stock of each member in such a way that it gives the reader, in a most interesting manner, a knowledge of the farm and conditions under which each herd is maintained, an intimate glimpse of the personality of the owner, a sample description of outstanding individual bulls and cows, the numerical strength of the herd and other details.

It is, as the author says, 'a brief factual presentation of the salient features and characteristics of the existing individual herds of pedigreed Scotch Shorthorns'. The interest of the book for Indian readers may well lie mostly in the exhibition of the skill of the author and the printers, for the breed is not one of those likely to suit us.

MG1PC—M—III-1-15—29-6-48—520.